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## **Some factors influencing the survival of microbial contamination in solid oral dosage forms.**

Blair, Tina Caroline

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SOME FACTORS INFLUENCING THE SURVIVAL OF  
MICROBIAL CONTAMINATION IN SOLID ORAL DOSAGE FORMS

A THESIS

Submitted in partial fulfilment of the  
requirements for the award of the degree of  
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## ABSTRACT

### Some factors influencing the survival of microbial contamination in solid oral dosage forms

The purpose of this work was to investigate the conditions in which bacteria survive, proliferate and die in solid oral dosage forms during processing and on subsequent storage. A method was developed for contaminating dry, directly-compressible tablet excipients with viable vegetative bacteria, resulting in the attachment of the bacteria to the powder particles.

The inactivation of bacteria during tableting of contaminated excipients was investigated. The levels of kill achieved depended on the compaction pressure applied, the organism size and shape, and the compaction mechanism of the formulation. Patterns of bacterial kill on tableting can illustrate the nature of particle deformation within the powder bed. Plastically-deforming excipients were most effective at killing bacteria at low compaction pressures.

Lubricants in tablet formulations may reduce microbial kill on compression, especially for fracturing materials. The efficiency of shearing forces in inactivating micro-organisms was shown by carrying out ram extrusion using a contaminated wet powder mass.

Contaminated tablets were stored in a range of relative humidity atmospheres at 25°C. The bacteria survived well at the lower relative humidities. Survival at higher relative humidities depended on the characteristics of the organisms, the relative humidity of storage, and the hygroscopicity of the excipients.

Differences in water-uptake characteristics between the excipients affected the responses of the attached bacteria to varying relative humidity conditions. Water availability to contaminating micro-organisms should therefore be described not only in terms of the partial vapour pressure of water in the environment, but also in terms of the hygroscopic properties of the solid substrate.

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For my parents, and for William and Robert.

Quando il gioco si fa duro,  
i duri cominciano a giocare.

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## 1. INTRODUCTION



## 1.1 THE CONTAMINATION OF SOLID ORAL DOSAGE FORMS

Historically, the dry nature of oral solid dosage forms has led to the assumption that such formulations are not at risk from microbial spoilage. However, a number of reported infection outbreaks associated with tablets and capsules indicate that this is not necessarily the case. Vegetative, pathogenic bacteria can survive in apparently dry pharmaceutical products, and some raw materials are more likely to be contaminated than others.

### 1.1.1 Incidences of contamination

A report by Kallings et al (1966a) to the Swedish National Board of Health first drew serious attention to the microbiological quality of non-sterile pharmaceuticals. This report described contaminated tablets containing numbers of coliforms "up to millions per gram". Publication of the report was closely followed by an outbreak of Salmonella muenchen infections in Sweden. This organism was sufficiently uncommon to warrant an epidemiological investigation into the outbreak. The infection was traced to defatted thyroid powder imported from Hungary, which contained over  $3 \times 10^7$  bacteria per gram, mostly faecal flora (Kallings et al, 1966b).

Lang et al (1967) and Eikhoff (1967) documented two epidemics of salmonellosis in the United States, involving one infant death. The infections were due to carmine powder contaminated with S. cubana. The powder, which was not infact intended for internal use, had been hand-filled into hard gelatin capsules and used as a

faecal dye. Serious incidents such as these prompted further surveys into the microbial content of solid oral dosage forms. In Denmark in 1968 Fischer et al and Fuglsang-Smidt and Ulrich examined the microbial content of a total of 696 batches of 237 types of tablets. Using non-specific viable counting techniques, they found that only 53 batches contained more than 100 bacteria per tablet, although of the three batches of thyroid tablets examined by Fischer et al (1968), counts as high as  $10^5$  organisms per tablet were found. Qualitative methods failed to identify the presence of either Salmonellae or E. coli.

In a survey of some antibiotic drugs, gross contamination of an antifungal powder by an aerobic spore-forming organism (Bacillus spp) was reported (White et al, 1968). Market samples of antibiotic preparations were examined by Bowman et al (1971), they found that of 44 batches of capsules and 5 batches of tablets, only 16 were contaminated. Of the contaminated batches, Staphylococcus epidermidis, the yeast Saccharomyces cerevisiae, Bacillus and Penicillium spp were present, but all samples were of "acceptable hygienic quality" since no batches contained more than 50 micro-organisms per gram, and tests for Pseudomonads, E. coli, Staphylococcus aureus (coagulase positive) and Salmonellae proved negative.

Gallien (1972) surveyed dosage forms produced in Europe by Hoffman-La Roche, and found 75% of samples to be of excellent microbiological quality, containing less than 10 organisms per gram. Where contamination was traced, it was generally less than



100 organisms per gram (fungi, Pseudomonads, Enterobacteriaceae), and clearly related to contamination of the raw materials used.

The microbiological quality of antacid tablets in India was studied by Jain and Chauhan (1978), revealing an appreciable level of contamination throughout the test batches, with a mean total viable count of  $4.36 \times 10^3$  per gram. Contamination levels were found to be higher for batches from small-scale manufacturers than from large-scale manufacturers, the consequences of inferior production facilities and control. A similar study of market samples of tablets showed almost all samples to contain fungal contamination, amongst which Candida albicans, Aspergillus niger and A. flavus were identified. Bacterial contamination was found to be up to  $1.16 \times 10^4$  per gram (Khante et al, 1979).

In a Rhodesian survey (Somerville, 1981) of non-sterile pharmaceuticals, 35 out of 174 samples showed growth, over half of those contaminated contained less than 100 organisms per gram. The tablets examined were found to be "microbiologically satisfactory", since potentially pathogenic organisms were not identified. However in a more recent survey, pathogenic bacteria were found in many of the tablets examined in samples taken from an Indian hospital pharmacy (Nandapurkar et al, 1985). Garcia-Arribas et al (1986) carried out an extensive study of contamination of oral solid dosage forms. Of 240 samples, over 30% were contaminated with aerobic bacteria, mostly Bacillus spp, of which 18 species were identified.

Table 1.1 summarises some reported incidences of contamination.

Table 1.1 Contamination reports for solid oral dosage forms

Report	Number of batches examined	Number of batches with the following numbers of viable organisms per gram					
		<10	10-10 <sup>2</sup>	10 <sup>2</sup> -10 <sup>3</sup>	10 <sup>3</sup> -10 <sup>4</sup>	10 <sup>4</sup> -10 <sup>5</sup>	10 <sup>5</sup> -10 <sup>6</sup>
Kallings <u>et al</u> , 1966b	157	119	4	6	8	4	16
Fuglsang- Smidt & Ulrich, 1968	360	247+	89	18	5	1	-
Fischer <u>et al</u> , 1968	336*	233	74	17	9	2	1
Bowman, 1971	49	34	15	0	0	-	-
Somerville, 1981	174	146	15	8	3	2	-
Garcia- Arribas <u>et al</u> , 1986	240	167+	36	21	16 (>10 <sup>3</sup> )		

\* pour plate counts

+ where 10<sup>1</sup> was the lower detection limit



### 1.1.2 Microbiological surveys of raw materials

Since solid oral dosage forms are dry, they offer an inhospitable environment for microbial proliferation. It is therefore unlikely that organisms introduced into dry dosage forms during processing will flourish: the major risk is rather that the indigenous population of contaminants present in the raw materials used will be able to persist into the final product, and survive on storage.

Surveys of microbial populations in pharmaceutical raw materials have revealed remarkably high counts in active drug substances and excipients alike. Pedersen and Ulrich (1968) examined 226 batches of 84 different raw materials. High viable counts ( $>10^5$  organisms per gram) were found in natural products such as agar, liquorice and digitalis leaf (one batch containing  $>10^7$  per gram). Salmonellae were absent but low levels of E. coli were found in 10 batches, including lactose, prednisolone, prednisone and talc.

Kallings et al (1966b) had stated that tests on a large number of raw materials showed starch to be the most commonly contaminated, and that potato or wheat starch were more at risk than maize starch. Similarly, Knusel and Hess (1968) found higher viable counts in wheat starch than maize starch and concluded that "the starches are mainly to blame for the high counts in tablets".

Wozniak (1971) found high viable counts in oral medicines derived from plants. Up to  $10^7$  organisms per gram were detected, 75% of samples contained Bacillus cereus. Coagulase-positive Staph. aureus was also frequently isolated. The most contaminated

products were pills and granules containing liquorice root, gelatin or starch.

In other surveys of the microbial contamination of pharmaceutical raw materials (Schiller et al, 1968; Kruger, 1973) natural products were found to have the highest counts, especially those of animal origin. Samples of lactose, pharmaceutical grade, were examined for Enterobacteriaceae (Harrewijn et al, 1972). The detectable counts never exceeded  $0.6 \times 10^3$  per gram, and 90% had less than  $10^2$  per gram, however Enterobacter spp were detected in 38.2% of isolates.

Sykes (1971) pointed out that the initial processing required to extract and purify natural raw materials may substantially reduce the original microbial load. However, animal-derived products are often quite labile in nature, and so are subjected to less rigorous treatments than plant products. Animal products are also more likely to be contaminated with human pathogens, thus overall they may constitute a greater microbiological hazard than other raw materials.

In a 1966 survey carried out by the United States F.D.A. (Lennington, 1967), a variety of Salmonella species were isolated from imported drug substances of animal origin, such as thyroid, pancreatin and edible gelatin (see table 1.2).

Table 1.2 Salmonella isolations from drug substances imported into the United States  
(after Lennington, 1967)

Product	Country of origin	Serotype
Thyroid powder	Argentina	<u>S. newport</u>
Thyroid powder	Argentina	<u>S. derby</u>
Thyroid powder	Italy	<u>S. newport</u>
Thyroid powder	Argentina	<u>S. bovis morbificans</u>
Thyroid powder	Uruguay	<u>S. anatum</u>
Thyroid powder	Canada	<u>S. anatum</u>
Thyroid powder	Canada	<u>S. anatum</u>
Thyroid powder	Denmark	<u>S. bareilly</u>
Thyroid powder	Italy	<u>S. anatum</u>
Thyroid powder	Argentina	<u>S. typhi-murium</u>
Thyroid powder	Netherlands	<u>S. typhi-murium</u>
Pancreatin powder	Canada	<u>S. tennessee</u>
Pancreatin powder	Canada	<u>S. anatum</u>
Edible gelatin	W. Germany	<u>S. senftenberg</u>
Lactalbumin	Netherlands	<u>S. cubana</u>



Raw materials can be subdivided into two categories, according to their stability during processing (Wallhausser, 1977):

(i) where the microbial count can be reduced by suitable treatments without damage to the product;

(ii) where no suitable methods for reduction of microbial count are available. This second group includes enzymes, plant and animal organ extracts.

### 1.1.3 Hazards from microbial contamination

Surveys of the microbiological quality of oral solid dosage forms have shown that a variety of dry products and raw materials may contain high numbers of bacteria and fungi. The contamination of a pharmaceutical product may result in infection or colonisation of the patient. Chemical or physical breakdown of the product may occur, leading for example to discolouration, a change in activity or an "off" smell or taste.

#### 1.1.3.1 The risk of microbial infection

Most of the bacterial and fungal species isolated from dry pharmaceutical products do not present a direct hazard of infection to the patient. However, the presence of known pathogens and bowel flora in dry finished products and their raw material has been shown in a number of surveys (Schiller, 1968; Pedersen and Ulrich, 1968; Sykes, 1971; Wozniak, 1971; Kruger, 1973; Nandapurkar et al, 1985). As discussed in section 1.1.1, pathogens in solid oral dosage forms can be the cause of human infection (Kallings et al 1966b) but it would be impractical and



unnecessary to require all such products to be sterile. The need for control is important, and in setting limits we must consider the information available on the degree of risk associated with taking contaminated products. For an oral solid dosage form, this may depend on a number of factors.

The risk of infection from a contaminated drug depends on the types and numbers of organisms present, their virulence, pathogenicity, and the immunological status of the patient (Flaum, 1978). The doses of orally-administered pathogens required to cause disease have been studied. McCullough and Eisele (1951) administered a variety of Salmonella species and strains to human volunteers, and showed that depending on the strain, between  $1.6 \times 10^5$  and  $2.5 \times 10^7$  organisms could be given as a dose, without causing illness. However, an outbreak of salmonellosis was traced by Coyle et al (1988) to home-made ice cream containing S. enteridis  $2.1-5 \times 10^4$  per gram, and reported outbreaks involving chocolate and cheddar cheese suggest that the infective dose was as little as 50-100, and less than 10 organisms respectively (Gill, 1983; Greenwood and Hooper, 1983; D'Aoust, 1985). E. coli was fed to healthy adult males (Ferguson and June, 1952),  $7 \times 10^6$  organisms gave only slight illness in 7 out of 10 subjects, with no definite diarrhoea. Similarly, Pseudomonas aeruginosa was administered up to  $2 \times 10^8$  with recovery of the organism in the stools but no signs of clinical illness (Buck and Cook, 1969).

Resistance to bacterial infection also depends on the immunological status of the patients. Reduced resistance to

infection may for example be associated with patients with pre-existing disease, those undergoing chemotherapy, geriatric or perinatal patients (Parker, M.T. 1972). A survey of Listeria monocytogenes infections in the United States in 1986-7, showed that one third of the victims were perinatal, and of the others, 88% had one or more pre-existing disease (Schwartz et al, 1988).

#### 1.1.3.2 Microbial toxins

In his review, Wallhausser (1977) drew attention to mycotoxins and bacterial toxins, which are produced by living micro-organisms and are capable of exerting poisonous effects at extremely low concentrations. It is considered that mycotoxins, and particularly the aflatoxins, because of the universal occurrence of Aspergillus flavus, probably represent the greatest hazard. According to Wallhausser, bacterial toxins are equally noxious but largely overlooked. Bacillus species are the most commonly-isolated microbial contaminants from pharmaceutical products (Garcia-Arribas et al, 1986). Although generally considered to be non-pathogenic, Bacillus species have been reported to mediate serious infections, associated with exotoxin production (Farrar, 1963; Turnbull et al, 1979). Furthermore, the majority of strains isolated from pharmaceuticals are resistant to many antimicrobial agents (Garcia-Arribas et al, 1988). These organisms are also common laboratory contaminants, so that Bacillus isolations may be incorrectly attributed to poor experimental technique. Consequently it is possible that infections with Bacillus involvement may be more common than the literature would suggest.



#### 1.1.3.3 Physicochemical changes due to microbial contamination

Chemical or physical changes may take place as a result of microbial colonization of a product. If raw materials of natural origin are used, a range of nutrients are introduced and the capacity for spoilage is increased. A range of active drug substances may be inactivated as a consequence of microbial growth: the production of salicylic acid from aspirin is well-known, and for example the transformation of steroids which has been observed around fungal colonies on the surface of steroid tablets (Beveridge, 1977).

Where contamination levels are high there may be detectable signs of colony growth, for example coloured colonies, or tastes and odours from by-products such as hydrogen sulphide, ammonia, "fishy" amines or ketones (Smart and Spooner, 1972). In addition, gaseous products produced during microbial metabolism may cause distension of foil or blister packaging.

#### 1.1.4 The control of microbiological quality of solid oral dosage forms

In the late 1960's, increasing numbers of reports of drug-borne infections associated with traditionally non-sterile products, led to recognition of the need for control of microbiological contamination and the establishment of microbiological standards for pharmaceutical products in general. In Britain, working parties of the Public Health Laboratory Service, and the



Pharmaceutical Society, reviewed product quality and made recommendations which were published in 1971.

The Pharmaceutical Society working party investigated microbial contamination in products for oral and topical use. The investigation covered the levels and types of micro-organisms present in pharmaceutical products, their significance, and means of reducing or preventing microbial growth. Total viable counts, and the detection of named pathogenic bacteria were carried out, for both raw materials and finished products. High total viable counts were found in some raw materials, and tablets containing raw materials of natural origin contained up to  $10^5$  organisms per tablet. In their report, the Pharmaceutical Society working party were not in favour of the imposition of limits for total microbial counts in non-sterile pharmaceuticals, but rather promoted the use of "total counts" during manufacture to indicate satisfactory raw materials and process hygiene. The working party further recommended that official requirements should be introduced for the absence of specific types of pathogenic bacteria, for example *Salmonellae*, *E. coli* etc.

In Britain, the United States and Europe, increasing concern over the safety of contaminated pharmaceuticals, and the effect of micro-organisms upon the quality and efficacy of medicinal products, resulted in the introduction and adoption of limits by manufacturers and official bodies for the microbial quality of tablets and their raw materials (Table 1.3).

Table 1.3 Microbial limits for solid pharmaceuticals

Specification	Material/Product	Requirements
British Pharmacopeia 1988	Limits for materials considered to be at risk e.g. gelatin digitalis	Absence of : <u>E. coli</u> <u>Salmonella</u> spp. <u>Pseudomonas</u> spp.
USP XXI	"nature and frequency of testing vary according to product" e.g. natural products	Absence of : <u>Salmonella</u> spp.
European Pharmacopoeia 1975	Oral solid	$<10^4$ bacteria per gram $<10^2$ moulds or yeasts per gram <u>E. coli</u> absent
F.I.P. (Pharm. Acta Helv. 50:285)	Oral solid	$10^3$ - $10^4$ aerobic bacteria per gram $<10^2$ moulds or yeasts per gram
UK licensed products	Raw materials and finished products	Absence of : <u>E. coli</u> <u>Ps. aeruginosa</u> <u>Salmonella</u> spp. <u>Staph. aureus</u> & other contaminants according to risk
Hospital manufactured products	Raw materials and products	Absence of : <u>E. coli</u> <u>Ps. aeruginosa</u> & water-borne spp. <u>Salmonella</u> spp. <u>Staph. aureus</u>

In defining microbial standards for non-sterile products, both the levels and types of contaminating organisms should be considered, and whether they are hazardous or acceptable. Bearing in mind the varying resistance of patients to infection by micro-organisms, it would seem reasonable to set limits such that products can be safely administered to the patients who are most at risk of infection, for example due to age, immunosuppression, or existing pathological state. However, our knowledge of the levels of ingested micro-organisms which may cause infection is limited. Therefore, it could reasonably be argued that "safe" limits are impossible to define. Official limits which have been laid down reflect to a certain extent the level of microbial quality assurance that is achievable, and the low incidences of infection associated with solid dosage forms over years of use.

In 1966, Kallings and his co-workers (Kallings et al, 1966b) recommended a general level of hygiene for all non-sterile pharmaceuticals, with an upper contamination limit of 100 bacteria per gram. They also suggested that for products, mainly of plant or animal origin, where there may be difficulties in achieving this standard, tests for the absence of coli and named pathogens should be applied. Dony and Gerard (1968) proposed the adoption of principles of testing for oral pharmaceuticals similar to those recommended for canned food products (Buttiaux and Mossel, 1957). Namely, the absence of pathogens, toxin-producing organisms such as Staphylococcus aureus or certain Clostridia spp, absence of organisms which would indicate faecal contamination, and a limit of  $< 1 \times 10^4$  per gram for saprophytes.



A detailed discussion of the microbial specifications applied to non-sterile pharmaceuticals is not within the scope of the present work: a number of authors have commented on and reviewed the various standards and guidelines (Sykes, 1971; Wallhausser, 1977; Ringertz and Ringertz, 1982; Price, 1984; Baird, 1988).

In brief, the standards for microbiological quality which have been adopted by compendia, licensing authorities and manufacturers (table 1.3) have been based on two requirements: the complete absence of specified micro-organisms, or a restriction on total numbers of permitted micro-organisms. It is recognised that this latter standard may be difficult to impose, since the size of a population of contaminants will vary according to the time or stage of processing at which the product is tested, and the method of testing. However, this approach has been adopted by the European Pharmacopoeia, amongst others. Limits on total numbers are also used extensively by manufacturers for "in house" quality monitoring of raw materials and finished products.

The 1970 revision of the United States Pharmacopoeia (USP) XVIII was the first pharmacopoeia to include microbial limit tests for pharmaceuticals. In Britain and the United States, compendial requirements developed over the last two decades have stipulated the absence of specified bacteria only for certain "at risk" products. For example, the British Pharmacopoeia considers digitalis to be among those products which should meet requirements for the absence of E. coli, Salmonellae,

Pseudomonads. The approach adopted by the USP XXI is that natural raw materials should be monitored closely, since they may harbour harmful micro-organisms which could persist into the finished product. The USP XXI also states that "the nature and frequency of testing vary according to product. The significance of micro-organisms in non-sterile pharmaceutical products should be evaluated in terms of the use of the product, the nature of the product and the potential hazard to the user. It is suggested that certain categories of products should be tested routinely for specified microbial contamination, e.g. natural plant, animal and some mineral products for Salmonella spp".

Non-sterile pharmaceutical products should meet these established standards for microbiological quality, both after production and during subsequent storage. In its original 1971 report, the Pharmaceutical Society working party indicated how microbial quality assurance could be achieved by attention paid to certain stages in manufacture, namely, the raw materials, equipment and environment, the manufacturing process, and packaging. These guidelines are now detailed in the "Guide to Good Pharmaceutical Manufacturing Practice, 1983 Edition". In addition to defining GMP, the Pharmaceutical Society working party recommended that the inclusion of antimicrobial preservatives should be considered where microbial growth is possible, and that the formulation should be such that the levels of contaminating organisms are reduced or growth prevented. Problems associated with chemical preservatives, such as possible toxicity, limited spectrum of activity, and manufacturers' reluctance to use "declarable" raw

materials, have led to increased emphasis on physical methods of antimicrobial preservation. For solid oral dosage forms, adequate preservation may be assured by understanding both the effect of processing on a population of natural contaminants, and microbial survival in dry conditions after manufacture.



## 1.2 WATER ACTIVITY AND MICROBIAL SURVIVAL IN SOLID ORAL DOSAGE FORMS

### 1.2.1 The basic concept of water activity

Micro-organisms will only grow in aqueous solutions where nutrient and other conditions are favourable. The growth solution may be extremely dilute, as in the case of distilled water (Bigger and Nelson, 1941), or potentially very concentrated, as in the case of solid and semi-solid substrates. Growth in solid dosage forms may be considered as a special case of growth in a concentrated solution. In studying microbial survival and growth, it is important to appreciate the properties of aqueous solutions as they effect the availability of water to micro-organisms.

Early research in this field centred around the food industry. It had been discovered that foods could be preserved by reducing their water content, and that spoilage proceeded more rapidly under humid conditions; the reasons for this were not well understood. It was, however, noted that spoilage can proceed in some systems of relatively low water content, for example sucrose containing 4% moisture (dry basis), whereas potato starch containing 24% moisture (dry basis) is perfectly stable (figures from Van den Berg and Bruin, 1981). From such observations it became clear that it was not the water content per se, but some other factor related to the state of constituent water which determined the probability of spoilage.

Scott (1957) considered the water requirements of micro-organisms

involved in the spoilage of foodstuffs. He introduced the term "water activity" ( $A_w$ ) as a fundamental property of aqueous solutions, relating the effect of a solute on the availability of water to the depression of vapour pressure. The basic concepts of water activity are also explained in reviews by Troller and Christian (1978) and Van den Berg and Bruin (1981).

When a solute is dissolved in an aqueous solution, the re-orientation of water molecules around the solute will result in a decrease in entropy. The increase in intermolecular forces around the water molecules is reflected by a lowering of the vapour pressure of the solution, the extent of which, for an "ideal" solute, is given by Raoult's law, which describes the reduction in vapour pressure due to the addition of a solute:-

$$\frac{P_o - P}{P_o} = \frac{n_1}{n_1 + n_2} \quad (1)$$

$P$  and  $P_o$  are the respective vapour pressures of the solution and solvent, and  $n_1$  and  $n_2$  are the numbers of moles of solute and solvent.

One kilogram of water contains 55.51 moles, one mole of an ideal solute dissolved in one kilogram of water will lower the vapour pressure by  $1 / (1 + 55.51)$  or 0.0177, i.e. by 1.77%.

Alternatively we can consider the relative vapour pressure of the solution compared with that of the pure solvent:-

$$\frac{P}{P_o} = \frac{n_2}{n_1 + n_2} \quad (2)$$

indicating that, for a one molal solution, the vapour pressure is  $55.51 / (1 + 55.1)$  or 0.9823, or 98.23% of pure water. For aqueous solutions, the relative vapour pressure is expressed as a percentage, as the equilibrium relative humidity (ERH):-

$$\text{ERH} = \frac{P}{P_o} \times 100\% = \frac{n_2}{n_1 + n_2} \times 100\% \quad (3)$$

For the one molal solution above, the vapour in equilibrium with the solution will have a relative humidity (RH) of 98.23%. Whereas RH is used to describe the status of the atmosphere surrounding a solution, water activity ( $A_w$ ) is a basic property of the solution itself, and is used to describe the availability of water for biological, physical and chemical changes. By definition :-

$$A_w = \frac{P}{P_o} \quad (4)$$

When the rates of evaporation and condensation are equal, then ERH and  $A_w$  are interchangeable, and  $A_w$  is a factor of 100 less than ERH. Tables of solutions specifying vapour pressures and water activities are available (Stokes and Robinson, 1949; Carr and Harris, 1949) as sources of reference to workers wishing to produce atmospheres of known RH for laboratory experiments.



For non-ideal solutes, the lowering of vapour pressure may be greater than predicted by Raoult's law, especially for electrolytes where the discrepancy increases with the number of ions generated per molecule. To calculate  $A_w$  for non-ideal solutions, a molal osmotic coefficient ( $\phi$ ) is used:-

$$\ln A_w = \frac{-\nu m \phi}{55.51} \quad (3)$$

$\nu$  is the number of ions generated by each molecule of solute ( $\nu = 1$  for non-electrolytes),  $m$  is the molal concentration. Tabulated values of  $\phi$  for electrolytes are given by Robinson and Stokes (1955).

#### 1.2.2 Microbial growth and survival as a function of $A_w$

Table 1.4 Lowest recorded  $A_w$  for growth of micro-organisms

Organism	Minimum $A_w$
Mucors	0.93
Gram-negative bacteria	0.90
Yeasts	0.88
Gram-positive bacteria	0.86
Moulds	0.80
Halophilic bacteria	0.75
Xerophilic moulds	0.61
Osmophilic yeasts	0.62

(after Labuza et al, 1972; Troller and Christian, 1978; Leech,

1988).

Micro-organisms may be grouped according to the characteristic limiting  $A_w$  below which they will not grow. This was recognised in early literature as reviewed by Scott (1957) and Mossel and Ingram (1955). Approximate lower limits for microbial growth are shown above (table 1.4), and more detailed figures are given in table 1.5.

#### 1.2.2.1 Xerophilic fungi

Yeasts and moulds may grow in more concentrated environments, or under conditions of lower humidity than bacteria. The groups most resistant to drought are the xerophilic moulds or osmophilic yeasts. These were collectively termed "xerophilic fungi" by Pitt (1975), and defined as all species of fungi, including yeasts, "capable of growth, under at least one set of environmental conditions, at a water activity below 0.85". Pitt surveyed the minimum reported  $A_w$  and temperature conditions for the growth of over 40 xerophilic fungi, and noted that the organisms collectively represented only eleven fungal genera. Aspergillus and Penicillium species make up a large proportion of the xerophiles listed by Pitt, and include mycotoxin-producing species. These ubiquitous organisms have created spoilage problems for solid pharmaceuticals, because of their ability to germinate and grow profusely at  $A_w$  below those permitting growth of other micro-organisms. Beveridge and Bendell (1988) stored commercial samples of tablets at high humidities (0.82 - 1.00 $A_w$ ), and found that all contaminants showing growth were Penicillium

species, except for green-spored Aspergillus on paracetamol tablets; no bacterial or yeast growth was observed.

In the survey by Pitt (1975), the most drought-tolerant species surveyed were Saccharomyces rouxii which has been observed to grow at 0.62Aw (von Schelhorn, 1950), and Monascus (Xeromyces) bisporus, which has been shown to germinate at 0.605Aw (Pitt and Christian, 1968), and can thus claim the lowest Aw at which fungal growth has been reported.

#### 1.2.2.2 Moulds

Scott's 1957 review summarized early findings concerning mould germination, growth and Aw. At each Aw there is a characteristic growth rate constant, which decreases as the Aw is reduced (Tomkins, 1929). For spore germination, it was reported that a reduction in Aw brought about a reduction in germination rate, and that each fungus has an optimum temperature at which the rate of germination is greatest. Bonner (1948) surveyed 58 fungal species and reported minimum Aw values for germination between which were 0.70 and 0.98Aw, and that minima for the various species were fairly evenly distributed within this Aw range. Galloway (1935) observed that of 20 Aspergillus cultures, three germinated at 0.75Aw, seven germinated at 0.80Aw and ten cultures germinated at 0.85Aw. For Penicillium the limits for germination were in the region of 0.80 to 0.90Aw. For Penicillium spp present as contaminants of commercial tablet samples, Beveridge and Bendell (1988) reported that lowering the Aw could extend the latent period before spore germination. This was similarly observed by



Heinzeler (1939), who noted that the delay in onset of germination was more pronounced for older cultures.

Ayerst (1969) noted species differences in relation to  $A_w$  and temperature optima for isolates of Aspergillus and Penicillium; however, all isolates showed the widest  $A_w$  tolerance at temperatures closest to their optimum. Similarly, Horner and Anagnostopoulos (1973) noted that at unfavourable  $A_w$ , growth inhibition could be enhanced by varying the temperature or, to a lesser extent, pH.

Since the growth of microbial contaminants is often a surface phenomenon, the interaction of  $A_w$  and oxygen tension is of interest. Fassihi and Parker (1977) found that for Penicillium spp, inactivation at low  $A_w$  (below 0.44) was enhanced under conditions of vacuum.

#### 1.2.2.3 Yeasts

Published data on the water requirements of yeasts are largely concerned with the so-called osmophilic strains which can grow at comparatively low  $A_w$ . For example, von Schelhorn (1950) showed the remarkable resistance of a strain of Saccharomyces rouxii which can grow slowly in fructose syrup at 0.62 $A_w$ . However, the growth of osmophilic yeast strains is highly dependent upon the total water content and the presence of specific types of solute in the substrate solution. For this reason, osmophilic yeasts are not a problem with solid dosage forms produced from dry powders.

Scott (1957) concluded that in general, non-osmophilic strains of yeast have a higher water requirement than moulds, and the minimum  $A_w$  for growth is a stable characteristic for a particular strain. Studies by Burcik (1950) of 14 yeast strains stored at humidities controlled by sodium chloride solutions indicated  $A_w$  minima varying between 0.88 and 0.91. Scott (1936) reported that as is the case for moulds, the germination of yeast spores shows an increase in lag time and a reduction in the rate of growth when  $A_w$  is reduced.

#### 1.2.2.4 Bacteria

The water relations of bacteria have been reviewed by Troller and Christian (1978). Table 1.5 includes data on the minimum tolerated water activities for the growth of some species of bacteria. Growth at the  $A_w$  minimum was in each case recorded when all other conditions (temperature, pH, oxygen tension, nutrients) were optimum. "Growth" may refer to spore germination or, in some cases, an extremely slow increase in the numbers of viable organisms. There are halophilic species of bacteria, which are tolerant of low  $A_w$  but incapable of growing at high  $A_w$ . The growth of halophilic bacteria can only take place in the presence of specific inorganic salts, of which NaCl is the most important. The spoilage of solid oral dosage forms by halophiles therefore is not a significant consideration.

Most non-halophilic bacteria have an optimum  $A_w$  for growth in the region of 0.997-0.980 $A_w$  (Troller and Christian, 1978). When grown within this  $A_w$  range, bacteria are extremely sensitive to small

Table 1.5 Published data on the approximate minimum levels of  $A_w$  permitting microbial growth at temperatures near optimal (after Troller & Christian, 1978)

Moulds	$A_w$
<i>Aspergillus flavus</i>	0.78
<i>A. niger</i>	0.77
<i>A. restrictus</i>	0.75
<i>A. versicolor</i>	0.78
<i>A. wentii</i>	0.84
<i>Chrysosporium fastidium</i>	0.69
<i>C. xerophilum</i>	0.71
<i>Eurotium (Aspergillus) amstelodami</i>	0.70
<i>E. repens</i>	0.71
<i>E. rubrum</i>	0.70
<i>Monascus (Xeromyces) bisporus</i>	0.61
<i>Mucor plumbeus</i>	0.93
<i>Paecilomyces variotii</i>	0.84
<i>Penicillium crysogenum</i>	0.79
<i>P. citrinum</i>	0.80
<i>P. frequentans</i>	0.81
<i>P. martensii</i>	0.79
<i>P. puberulum</i>	0.81
<i>P. viridicatum</i>	0.81
<i>Rhizopus nigricans</i>	0.93
Yeasts	$A_w$
<i>Debaryomyces hansenii</i>	0.83
<i>Saccharomyces bailii</i>	0.80
<i>S. cerevisiae</i>	0.90
<i>S. rouxii</i>	0.62



Table 1.5, continued.

Bacteria	Aw
Aerobacter aerogenes	0.94
B. cereus	0.95
B. megaterium	0.95
B. stearthermophilus	0.93
B. subtilis	0.90
Clostridium botulinum type A	0.95
C. botulinum type B	0.94
C. botulinum type E	0.97
Escherichia coli	0.95
Halobacterium halobium	0.75
Staphylococcus aureus	0.86*
Vibrio costicolus	0.86

\* Tatini (1973) cites a minimum water activity for staphylococcal growth of 0.83Aw (Hill, W.M., 1973).

changes in  $A_w$ . It is therefore suggested that more dilute laboratory media (for example, nutrient broth, which has an  $A_w$  of 0.999) may provide suboptimal conditions for growth (Christian and Waltho, 1962). Troller and Christian (1978) reported that the Gram-negative species are most sensitive to  $A_w$ , with minima for growth in the range 0.96-0.94 $A_w$ . Bacillus species will grow at water activities between 0.93 and 0.90 $A_w$ . The widest  $A_w$  tolerance is shown by the gram-positive cocci for which  $A_w$  minima have been reported between 0.95 and 0.83 $A_w$ . Bacterial growth curves show increased lag time as the  $A_w$  decreases away from optimum; under the same conditions, the overall growth rate decreases, and a plot of growth rate versus  $A_w$  will give a straight line of negative slope.

The availability of oxygen to bacteria affects their tolerance of non-ideal  $A_w$  conditions. The death of bacterial cells at suboptimal  $A_w$  is enhanced by the availability of oxygen (Mossel, 1975). However, when studying the water requirements of Staphylococcus aureus, Scott (1953) reported growth in aerobic conditions from at  $A_w$  levels from 0.99 $A_w$  down to 0.86 $A_w$ , but in anaerobic conditions only down to 0.94 $A_w$ .

The minimum  $A_w$  for enterotoxin production by strains of Staph. aureus is higher than the minimum  $A_w$  for growth (Troller and Stinson, 1975). By reducing the water activity from 0.99 to 0.98 or 0.97 $A_w$ , enterotoxin B output was reduced by 90-99%, with minimal reduction in numbers of viable bacteria (Troller, 1971). Enterotoxin A-producing strains were less sensitive to changes in

$A_w$  than strains producing enterotoxin B. Nevertheless, a drop in water activity from 0.99 to 0.945 $A_w$  brought about a 55-60% reduction in the amount of enterotoxin A produced (Troller, 1972). These studies may explain why relatively large numbers of enterotoxin-producing Staph. aureus may be isolated from spoiled products, with no detectable enterotoxin.

The staphylococci and micrococci are poor competitors, and are rapidly overgrown by other organisms when grown in moist conditions. Their relative importance as food spoilage organisms is due to their ability to grow in relatively dry or concentrated environments. Once microbial growth has been initiated by such organisms, metabolically produced water will raise local  $A_w$  levels, further accelerating the growth rate and allowing previously inhibited organisms to grow (Mossel, 1975; Beveridge, 1977). The way in which the staphylococci and micrococci tolerate low  $A_w$  has been studied. Christian and Waltho (1962) found a correlation between increased uptake of inorganic salts and the ability to grow at low  $A_w$ , for strains of staphylococci and micrococci in media containing low molecular weight salts. It was postulated that the accumulation of salts enabled the bacteria to rapidly equilibrate with the surround medium, and modified their responses to changing  $A_w$ , and other external factors.

Calhoun and Frazier (1966) subjected bacteria to heating in solutions of controlled  $A_w$ . They found that the thermal resistance of bacteria depended on whether an ionising (NaCl) or non-ionising (glucose) solute had been used to adjust the  $A_w$  in



the growth or heating menstruum. They also found that solute diffusion into Gram-positive organisms was more rapid than for Gram-negatives. In a review of the water requirements of food-borne bacterial pathogens, Troller (1973) cited work demonstrating the effect of solutes on the  $A_w$  tolerance of bacteria. Baird-Parker and Freame (1967) found that the minimum  $A_w$  for the germination of Clostridium botulinum spores varied according to the solute chosen to adjust  $A_w$ . Spore germination was achieved at 0.93 $A_w$  using NaCl, or 0.89 $A_w$  if glycerol was the adjusting solute. For C. perfringens, growth was achieved between 0.97 $A_w$  and 0.95 $A_w$  using NaCl or KCl, or 0.95 $A_w$  to 0.93 $A_w$  using glycerol (Kang et al, 1969). For Salmonella oranienburg, Christian (1955) observed growth at 0.96 $A_w$  in glycerol, but at 0.97 $A_w$  in other solutes. These results show that the responses of bacteria to reduced  $A_w$ , associated with the addition of solutes or the removal of water, are not due solely to the reduction in  $A_w$ , but also depend on the types of solutes present.

In  $A_w$  conditions too dry or concentrated for growth,  $A_w$  is still an important factor in determining the survival of a bacterial population of vegetative cells or spores. For cultures of Streptococcus agalactiae dried in milk, it was found that the bacterial death rate was dependent upon the humidity of storage (Watts, 1945). However, unlike the relationship between growth rate and  $A_w$ , the relationship between death rate and  $A_w$  was not linear, and at each particular  $A_w$  the death rate was not logarithmic. In the same study, maximum bacterial survival was shown by cultures stored at  $A_w$  values between 0.15 and 0.25, with

death rates increased when cultures were stored between 0.25Aw and 0.75Aw. In another study, spores and dried vegetative cells were stored in atmospheres controlled by sulphuric acid solutions. Survival was good between 0.05 and 0.20Aw, with best survival at 0.10Aw. At higher Aw values, between 0.80 and 1.00Aw, viable counts fell initially and then began to increase, until overgrown by moulds. Survival decreased as the Aw was reduced below 1.00Aw, and then increased as the Aw approached 0.10 (Higginbottom, 1953). Similarly, Scott (1958) found that the survival of dried cultures including S. aureus, in vacuo was better at 0.07, 0.11 and 0.16Aw than at 0.00 or 0.22Aw, and dried cultures of Salmonella newport in air, a slightly higher Aw, of 0.20 was recorded.

From the available information on the relationship between Aw and microbial survival, it is suggested that at the Aw values normally associated with pharmaceutical solid dosage forms, the survival of both vegetative and spore-forming organisms alike may be expected. On increasing the storage humidity, it is possible that organisms more tolerant of drier conditions could begin to grow and metabolise, releasing more water to initiate the growth of other organisms. However, it is also possible that by increasing water activity, active growth may be initiated in an environment which is too concentrated for the micro-organisms to survive, resulting in death of the whole population.

### 1.3 THE WATER VAPOUR - SOLID INTERACTION

#### 1.3.1 Hygroscopicity and equilibrium moisture content

The amount and character of water held by a "dry" material at a particular  $A_w$  is described in terms of the hygroscopic nature of the material involved.

The equilibrium moisture content (EMC) is achieved when the vapour pressure due to the moisture content of the solid is the same as the partial pressure of the water vapour in the air. A knowledge of the EMC of excipients is necessary for formulating moisture-stable products, and for specifying proper drying and storage conditions. The EMC of a powder mix will be the weighted mean of the EMC values of the various components, and each can be predicted for different water activities of interest (Scott et al, 1963).

Pharmaceutical excipients may be classified into four categories of hygroscopicity (see figures 1.1 and 1.2) according to measured EMC at a range of  $A_w$  (Callahan et al, 1982):

Class I - non-hygroscopic, for example lactose monohydrate;

Class II - slightly hygroscopic, for example microcrystalline cellulose;

Class III - moderately hygroscopic, for example maize starch;

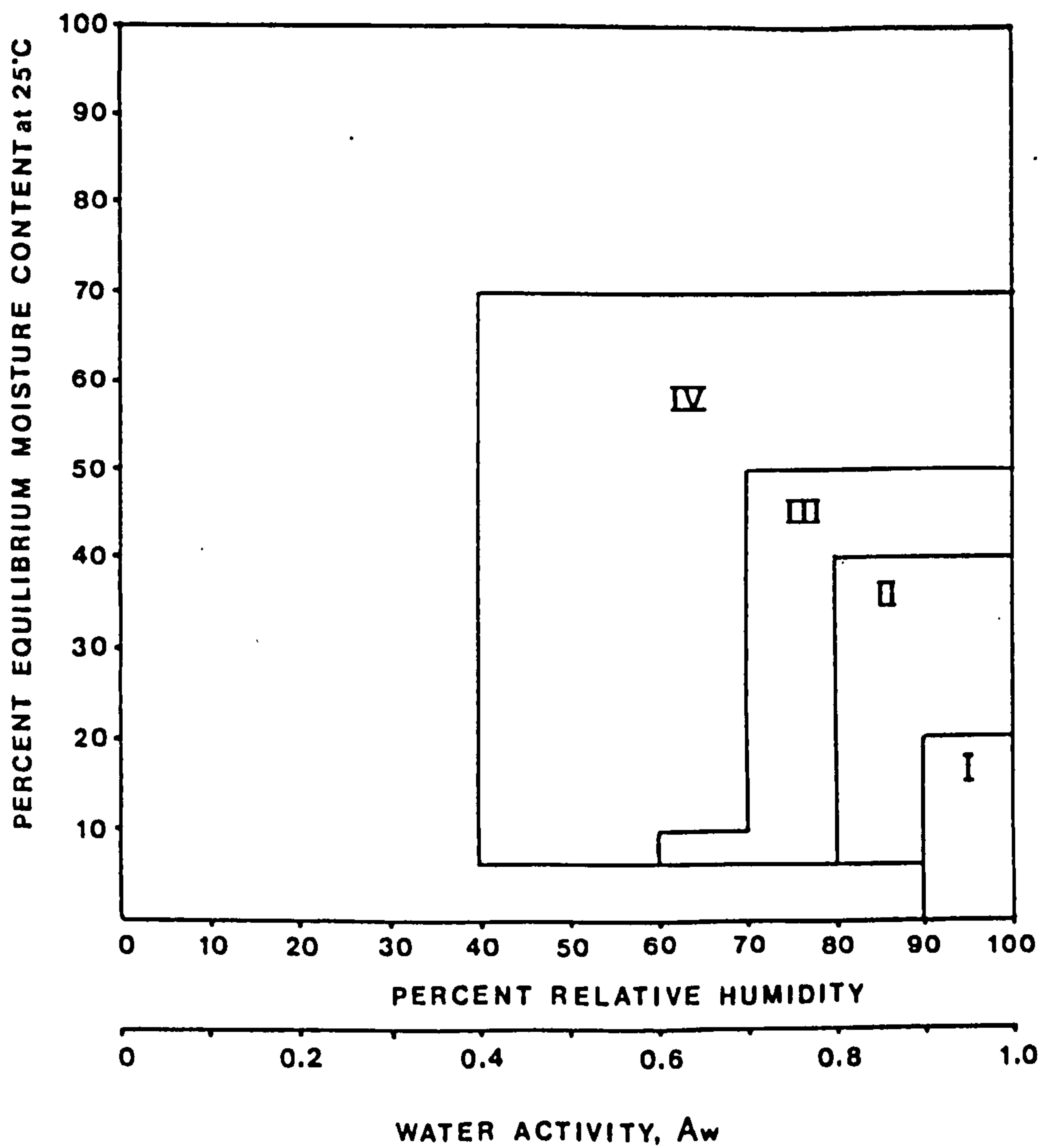
Class IV - very hygroscopic, for example sodium carboxymethyl-cellulose.

It was noted by the authors (Callahan et al, 1982) that excipients



Figure 1.1 Hygroscopicity classification

(after Callahan et al, 1982).



Class I - non-hygroscopic

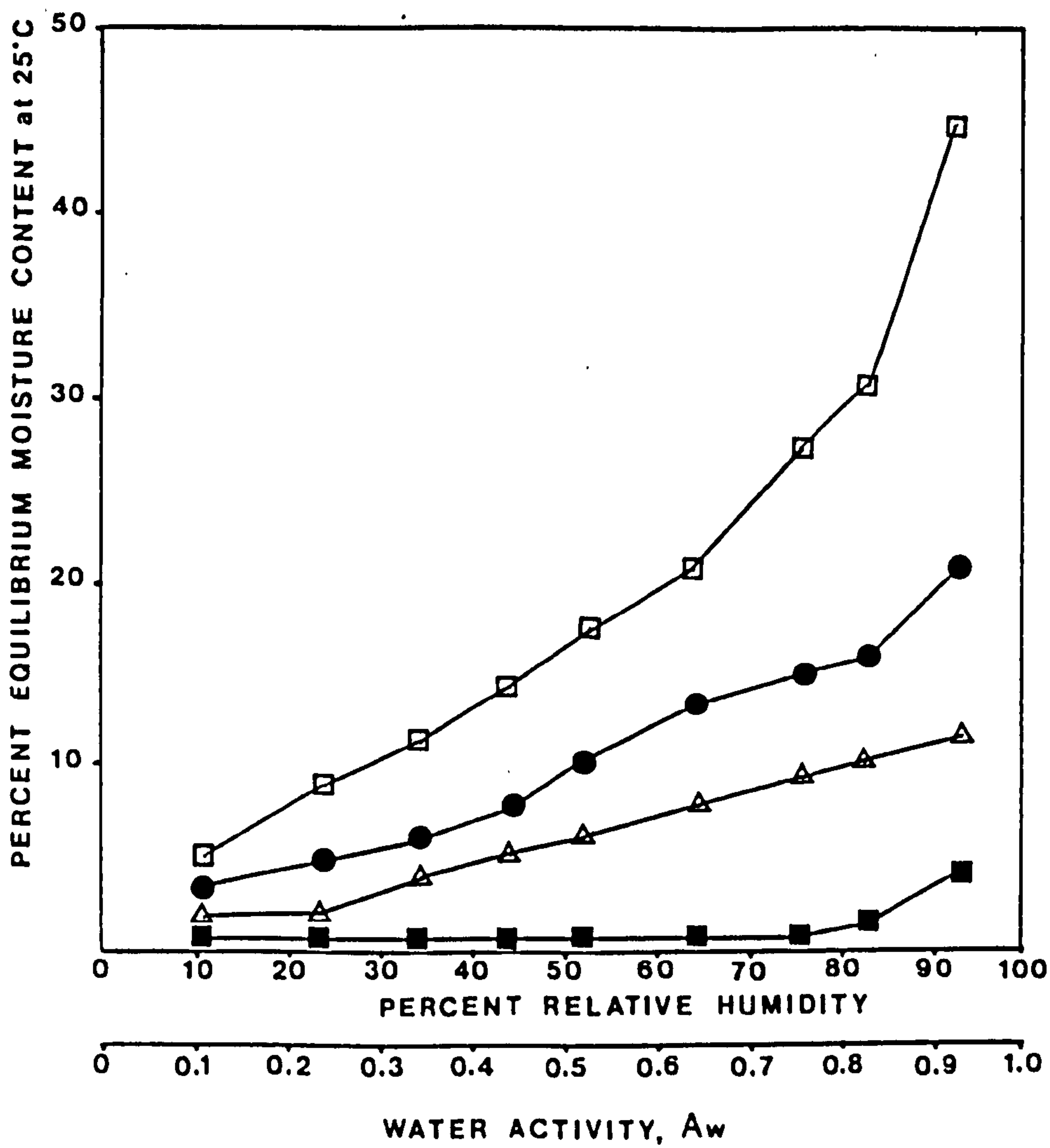
Class II - slightly hygroscopic

Class III - moderately hygroscopic

Class IV - very hygroscopic

Figure 1.2 EMC curves for representative excipients according to hygroscopic classification

(after Callahan et al, 1982).



- Class I (anhydrous lactose USP)
- △ Class II (cellulose acetate phthalate NF)
- Class III (magnesium aluminium silicate NF)
- Class IV (povidone USP)

with a high EMC classification may require special handling and packaging, and in some cases, chemical modification to reduce water uptake.

The term "hygroscopicity" relates to both the EMC of a substance and the rate at which it is achieved. Various workers have attempted to define hygroscopicity in thermodynamic or kinetic terms, as reviewed by Van Campen et al (1980). Markowitz and Boryta (1961) chose as the criterion for hygroscopicity "the negative free energy change for the isothermal transfer of one mole of water from the vapour pressure of pure water to the vapour pressure of the system under test". This value of  $-\Delta G$  they termed the "hygroscopicity potential", HP:

$$HP = -\Delta G = \mu_{H_2O_{\text{pure}}} - \mu_{H_2O_{\text{system}}} \quad (6)$$

$$\text{as } \Delta G^\ominus = -RT \ln(K) \quad (7)$$

$$HP = RT \ln( P_o / P ) \quad (8)$$

R = the gas constant

T = absolute temperature

$\mu$  = the chemical potential for each system.

$P_o$  = saturated vapour pressure

P = vapour pressure

K = equilibrium constant

$\ominus$  = indication of a defined standard state

The kinetic element of hygroscopicity has been described by Kuvshinnikov et al (1971) using a "hygroscopicity coefficient",





defined as the logarithm of the initial slope of the kinetic curve of moisture uptake:

$$W = W_{eq} (1 - e^{-kt}) \quad (9)$$

$W$  = moisture content

$W_{eq}$  = the equilibrium moisture content (EMC)

$k$  = a constant of uptake

$t$  = time

### 1.3.2 Water sorption isotherms and water binding

The reversible combination of water with a solid is described as sorption (humidification) and desorption (drying). Water vapour sorption may involve both real physical and chemical adsorption, as well as absorption by the solid.

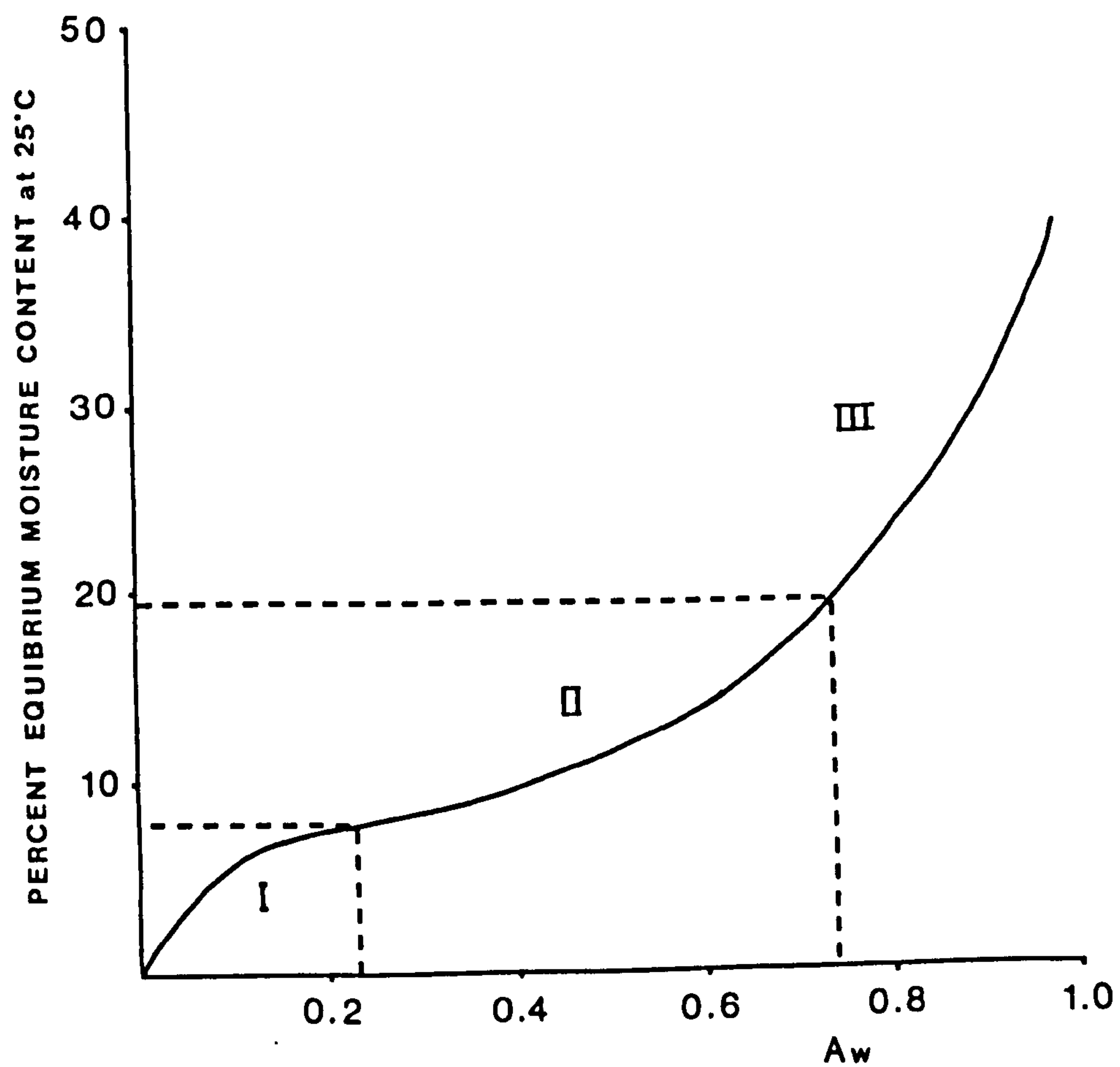
Sorption isotherms give a graphical representation of moisture content varying with the partial vapour pressure of water in the surrounding atmosphere: they can be presented as isothermal plots of EMC (usually determined gravimetrically) versus pressure or a pressure function such as RH or  $A_w$ .

Isotherms may be used by the formulator to predict the effect of changes in moisture content on  $A_w$  levels; it is then possible to predict the levels of moisture content at which desirable or undesirable effects due to  $A_w$  are likely to occur.

A generalized water vapour sorption isotherm is shown in figure 1.3 (after Van den Berg and Bruin, 1981). The three regions of the curve represent the differing ways in which water interacts

Figure 1.3 Generalized water vapour sorption isotherm of a food system, showing three ranges of sorption

(after Van den Berg and Bruin, 1981).



with the substrate, according to the  $A_w$ . Region I represents strongly bound water, which may have an enthalpy of vaporization much higher than that of pure water. This water is regarded as a sub-monolayer coverage and is bonded directly onto the powder. If the powder/water bonding is favoured, then water in region I behaves essentially as part of the solid and is unavailable for reactions. In region II, it is often considered that a monolayer coverage has been achieved, subsequent water molecules build up on top of those in the first region as multilayers. The enthalpy of vaporization is little higher than that of pure water. The BET isotherm (Brunauer et al, 1938) states that the first layer of bound water molecules are most indicative of the powder/water interaction, and as such may be very strongly bound. As more layers of water molecules bind to the surface the influence of the powder rapidly becomes less and less until after about two or three molecules of water the layers can be considered as condensed water. These water molecules are more available to take part in chemical and biological reactions, or to act as a solvent. Lastly, region III shows water mechanically trapped by void spaces, with little or no detectable enthalpy of adsorption. This water is unfreezable at  $0^{\circ}\text{C}$  and has a  $A_w$  of less than 1.00, nevertheless it has many properties similar to those of bulk water, and is relatively available for chemical and biological processes.

In certain materials, the amount of water sorbed at a given  $A_w$  and temperature may vary according to whether the moisture content is achieved by sorption from a dry state, or by desorption (see



Figure 1.4 Moisture vapour adsorption and desorption isotherms, showing hysteresis, for hard gelatin capsules at 25°C  
(after York, 1981).

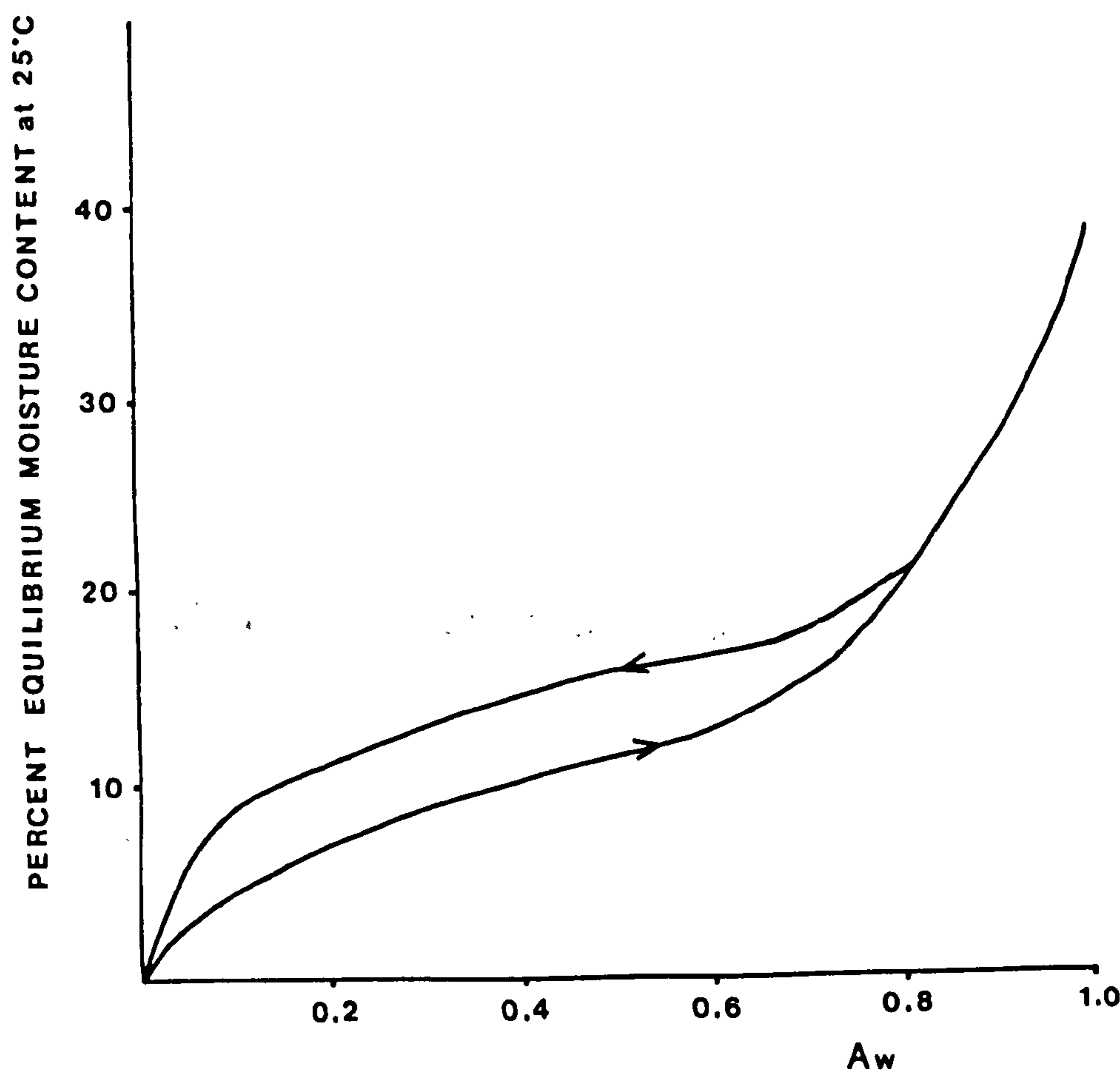


figure 1.4). This effect is termed hysteresis and is discussed below in further detail.

#### 1.3.2.1 The characterization of water sorption isotherms

The shape and magnitude of an isotherm depend on the nature of the association between water and substrate across the  $A_w$  range. This relationship reflects the morphology of powder particles and the energetics of their surfaces (Schroder, 1984). Isotherm shapes may be classified into five types (figure 1.5), according to the vapour-substrate interaction (Brunauer et al, 1940). Relatively hygroscopic pharmaceutical excipients commonly show a Type II sigmoidal curve. This shape results from a multimolecular build-up of adsorbed vapour, giving a point of inflection or plateau when the first monomolecular layer nears completion.

An early model for characterizing the first part of the isotherm, where there is adsorption of a monolayer of vapour onto the solid substrate, was given by Langmuir (1918):

$$V / V_m = bA_w / 1 + bA_w \quad (10)$$

where  $V$  = volume adsorbed per gram of solid

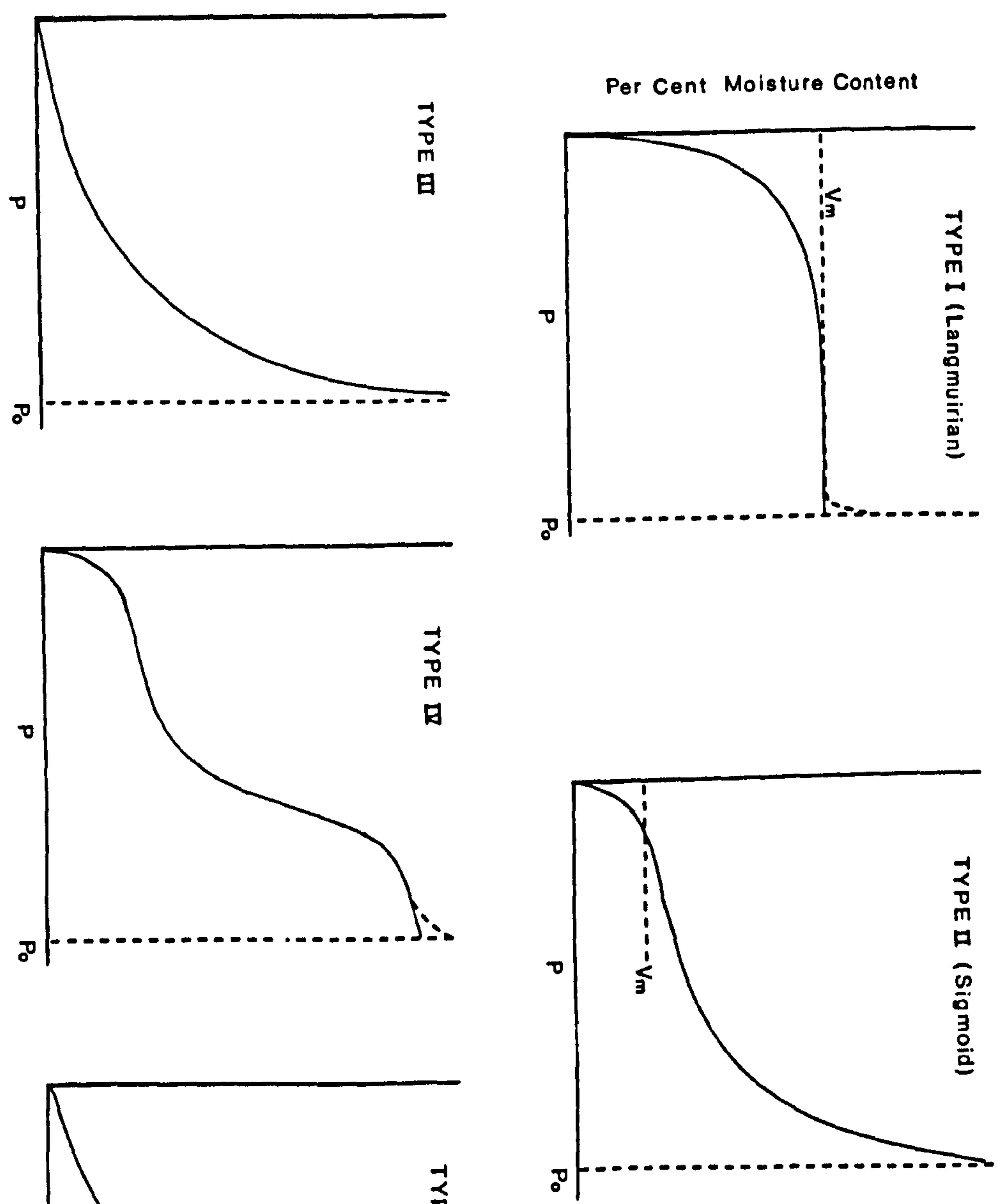
$V_m$  = monolayer volume per gram of solid

$b$  = a constant (temperature dependent)

$A_w$  = water activity

This may be rearranged (equation 11) so that a plot of  $A_w / V$  against  $A_w$  gives a straight line with a slope of  $1 / V_m$ , in order to calculate the monolayer:

Figure 1.5 Classification of physical adsorption isotherms



$P$  = vapour pressure  
 $P_0$  = saturated vapour pressure  
y axis = moisture content as a percentage of dry weight



$$A_w / V = [ 1 / bV_m ] + [A_w / V_m] \quad (11)$$

The relation is limited by its assumption that adsorption sites are homogeneously dispersed across the surface, and that all sites are equally accessible, consequently this isotherm does not account for interaction between adsorbed molecules (Langmuir, 1918). For many systems, multilayer adsorption occurs, so that above 0.25  $A_w$  the relationship fails (Tabibi and Hollenbeck, 1984). When considering multilayer adsorption, a more useful isotherm is the BET model (Brunauer et al, 1938):

$$V / V_m = c A_w / [1 - A_w][1 + (c + 1)A_w] \quad (12)$$

This assumes sorption at specific sites, and that the heat of sorption after the monolayer tends to the heat of vaporization. The relation gives an s-shaped curve (type II) when the volume sorbed is plotted against  $A_w$ , and may also be written thus:

$$[A_w / (1 - A_w)V] = [1 / V_m c] + [A_w(c - 1) / V_m c] \quad (13)$$

so that a plot of  $A_w / (1 - A_w)V$  against  $A_w$  gives a straight line of slope  $[c - 1 / c V_m]$ , with intercept  $1 / c V_m$ .

$c$  is the BET constant, related to the heat of binding for the system involved:

$$c = g \exp(\Delta H_{\text{mon}} - \Delta H_{\text{cond}} / RT) \quad (14)$$

where  $\Delta H_{\text{mon}}$  = the enthalpy of adsorption of the monolayer

$\Delta H_{\text{cond}}$  = the enthalpy of condensation of water.

The difference between  $\Delta H_{\text{mon}}$  and  $\Delta H_{\text{cond}}$  may be called the

enthalpy of binding,  $\Delta_{HB}$ , so that :

$$c = g \exp( \Delta_{HB} / RT) \quad (15)$$

and  $g$  is a constant, related to the proportion of surface area occupied by various numbers of layers of adsorbate.

The BET isotherm model can be used to calculate the monolayer capacity, from which the effective surface area available for water adsorption can be deduced (Gupta and Bhatia, 1969; Zografis *et al*, 1984; Wurster *et al*, 1982).

However, the model assumes that the influence of the solid on adsorption does not extend past the first layer, and that subsequent layers behave as bulk water. As a result, the model does not apply to many systems above 0.35-0.40 $A_w$  (Van den Berg and Bruin, 1981; Zografis, 1988).

An expression which takes into account an intermediate thermodynamic state, between monolayer-bound and condensed water, has been derived from the BET equation. Known as the GAB equation, it was developed independently by Guggenheim (1966), Anderson (1946) and deBoer (1968):

$$V = V_m C_g i A_w / [1 - i A_w][1 - i A_w + C_g A_w] \quad (16)$$

$C_g$  and  $i$  are constants,  $C_g$  relating to the free energy of sorption, where the second constant,  $i$ , relates to the solid-water interaction for layers on top of the monolayer. The GAB equation has been found to fit sorption isotherms well, in many cases over

the whole  $A_w$  range (Zograf, 1988; Kontny, 1988).

In addition to relationships applied to single isotherms, the Clausius-Clapeyron equation uses multitemperature water adsorption isotherms to give information on the portion of water bound to specific polar sites (Adamson, 1967; Zettlemoyer et al, 1975; Karel, 1973). A derived form of the equation, assuming fixed values for the  $A_w$  and moisture content, is given below (equation 17):

$$\ln A_w = (\Delta H_B / R)(1 / T) + q \quad (17)$$

where  $H_B$  = enthalpy of binding

$R$  = the gas constant

$T$  = temperature

$q$  = a constant

For a constant water content, a plot of  $\ln(A_w)$  versus  $1 / T$  gives a straight line, with slope  $\Delta H_B / R$ . This has been used to calculate the enthalpy of adsorption for water onto powders of pharmaceutical interest (Shotton and Harb, 1965) and foods (Soekarto and Steinberg, 1981). However, the calculation of an isosteric enthalpy of adsorption, using the temperature dependence of an equilibrium constant, should be limited to systems where  $\Delta H_B$  is constant over the temperature range investigated, and isotherms are truly reversible with respect to temperature and pressure (Van Campen et al, 1980). Otherwise, direct and sensitive calorimetric measurements are preferable.



#### 1.3.2.2 Sorption hysteresis

An example of a hysteresis loop is shown in figure 1.4. Hysteresis is the effect whereby a higher moisture content is achieved on desorption than at the same  $A_w$  on sorption. The precise causes of hysteresis are debatable, and depend on the nature of material involved.

For porous materials hysteresis may be explained by capillary condensation effects (Foster, 1951; Labuza and Rutman, 1968). These arise when the surface of a liquid is highly curved in a capillary, resulting in a change in the partial vapour pressure, as given by the Kelvin equation:

$$\ln A_w = \ln P / P_o = (-2\gamma \cos \theta V_w) / r RT \quad (18)$$

where  $\gamma$  = the liquid-air interfacial tension

$\theta$  = contact angle

$V_w$  = molar volume of water

$r$  = capillary radius

$R$  = gas constant

$T$  = absolute temperature

A higher contact angle is assumed for the sorption curve of the isotherm, so that at a given moisture content, the  $A_w$  will be higher on sorption than on desorption. However, this should only occur in region III of the isotherm (see figure 1.3), where the condensate has "bulk water" properties, whereas the loop may be extended further, in some cases beyond the monolayer.

There is also the "open pore" theory (Foster, 1951), whereby

capillary condensation on adsorption depends upon the formation of a cylindrical meniscus around the inside of the capillary. In this case, hysteresis depends upon pore size: for narrower pores the meniscus forms without delay giving no hysteresis.

In some cases, hysteresis may be a rate function, which diminishes as true equilibrium is approached. Rao (1941) proposed the "ink-bottle" model, in which hysteresis results from capillaries having large cavities and narrow necks. It is suggested that the sorption curve represents the true equilibrium state, and on desorption, the exit of vapour molecules is hindered by the lower vapour pressure in the narrow neck. This theory is supported by the findings of other workers (Gupta and Bhatia, 1969; Labuza and Rutman, 1968).

However, hysteresis is also shown by systems which are thermodynamically stable, reflecting an essential difference in the free energies of the sorption and desorption branches of the isotherm. Hollenbeck et al (1978) investigated the water-microcrystalline cellulose system. They explained that at equilibrium, hysteresis may reflect differences in specific surface area due to irreversible swelling, enthalpy effects due to differences in solid-liquid bonding, or entropy effects due to changes in molecular ordering.

For biological materials, Young and Nelson (1967a), derived isotherm equations in the same way as Langmuir (1918) and Brunauer et al (1938), adding a contribution from absorbed water. They

proposed that water is taken up in three ways: (i) as monolayer adsorbed moisture; (ii) as moisture absorbed within the material; (iii) as multimolecular (condensed) layers of moisture. On sorption water is driven by diffusional forces from the monomolecular outer layer to the inside of the cell, whereas on desorption, this bound water on the outer surface must be removed before diffusion from inside the cell can take place, resulting in hysteresis.

The relevant equations for total water sorption,  $M_s$ , and for desorption,  $M_d$ , are:

$$M_s = A (Y + \alpha) + B f \quad (19)$$

$$M_d = A (Y + \alpha) + B Y (A w_{\max}) \quad (20)$$

$Y$  = fraction of surface covered by a monolayer of moisture

$f$  = fraction of surface covered by at least one layer of normally condensed moisture on top of bound moisture

$\alpha$  = number of molecular layers of condensed moisture

$$A = \rho V_m / M \quad (19a)$$

$$B = \rho V / M \quad (20a)$$

where  $\rho$  = density of water

$V_m$  = volume of water in the monolayer

$V$  = amount of sorbed moisture at saturation

$M$  = mass of dry material

These equations (19 and 20) were found to fit adsorption processes for wheat (Young and Nelson, 1967b), and were used to characterize isotherms of gelatin, maize starch and maize starch:barbital



mixtures (York, 1981). However, they do not take into account two possible sets of  $V_m / M$  values for materials where hysteresis occurs at low  $A_w$  (Zograf et al, 1983). Thus the application of the Young and Nelson equations to materials such as starch may not be justified.

An explanation for the hysteresis shown by starches, that of reversible hydrogen bond breakage, is supported by the findings of Sair and Fetzer (1944) and Wurster et al (1982). It is proposed that on drying, hydrogen bonds form between the starch hydroxyl groups. On exposure to moisture, any remaining free hydroxyl groups will sorb water first, and adsorption is then regulated by the gradual replacement of the inter-hydroxyl bonds by hydroxyl-water bonds. This theory allows for changes in available surface area due to swelling on adsorption.

Zograf et al (1984) propose that water sorbed to polymers such as starch and cellulose exists in three states: tightly bound to an anhydroglucose unit; less tightly bound; and bulk water. They believe that for starch, water sorption does not rely on a pre-existing pore structure as suggested by others (Gupta and Bhatia, 1969; Wurster et al, 1982), but occurs through vapour penetration of the solid. Water penetrates throughout the starch grain, and strong hydration takes place with changes in polymer conformation and swelling. This explains, for example, why BET monolayer values for different types of starch agree closely: if porosity governed sorption, then different grain morphologies should give significantly different monolayer results.

For celluloses, it is proposed that water uptake takes place in very much the same way as for starch, but only in the amorphous or non-crystalline portion. Thus milling microcrystalline cellulose enhances its water-sorbing ability, not by increasing available surface area, but by reducing the degree of crystallinity (Nakai et al, 1977; Zografi et al, 1984). Similarly, processing of microcrystalline cellulose into derivatives such as sodium carboxymethylcellulose, eliminates the crystallinity of cellulose and results in very hygroscopic materials (Zografi and Kontny, 1986).

## 1.4 METHODS FOR THE DETERMINATION OF SORBED WATER

### 1.4.1 Total water content

The total water content in a solid sample is generally determined by thermal dehydration, and the evolved water by Karl Fischer titration.

Sorption isotherms may be constructed from direct gravimetric measurements of solids exposed to known water vapour pressures. Most simply, this involves the equilibration of solid samples with an environment of controlled temperature and relative humidity. A faster and more sophisticated method is to use a vacuum microbalance system, as reviewed by Czanderna and Vasofsky (1979), and applied to water vapour sorption by Jacobasch (1979) and Buckton et al (1986).

### 1.4.2 Spectroscopic methods

Methods to determine the reactivity or mobility of sorbed water give information on how the water is bound. For example, spectroscopic methods have been used to monitor the way in which various types of electromagnetic radiation interact with sorbed water, and were reviewed by Brittain et al (1988).

Wide line proton magnetic resonance is used routinely to determine the content of water in foods. Molecules of water in the liquid state move randomly, and all experience the same net field when exposed to a magnet. On exposure to radio frequency radiation, these molecules will absorb energy at a similar frequency, giving



a sharp difference between received and transmitted radio signal. However, the proton response given by solid water will be influenced by neighbouring atoms, giving a broader, more shallow difference in radio signal. In this way, bound water can be distinguished using NMR by a signal intermediate between liquid and solid water (Karel, 1973; Duckworth, 1972). The contribution by -OH groups not associated with water can be excluded by comparing measurements with those from a dehydrated sample. NMR was used to determine the hydrated states of ampicillin by Brittain et al (1988). Water binding on starches was studied by application of NMR by Lechert (1981), and NMR was used to determine the state of water in foods (Nagashima and Suzuki, 1981; Duckworth, 1972; Steinberg and Leung, 1975). Progressive uptake of moisture by stored digitalis tablets, was monitored with proton pulse NMR by Beveridge and Bendell (1988).

Using infrared spectroscopy, the -OH stretching frequency of water can be measured; for free (gaseous) water this frequency is characteristic, and is reduced by water binding to a solid. By examination of the -OH band associated with water, a distinction can be made between bound, trapped or surface condensed water.

#### 1.4.3 Dielectric response

Water bound by polymers, proteins and foods can also be estimated using dielectric response measurements. Bound water will show dielectric properties intermediate between those of solid water and more mobile liquid water, depending on the molecules in the immediate environment (Karel, 1973). Khan and Pilpel (1987)

monitored a type of dielectric behaviour called "low frequency dispersion", as water was sorbed to microcrystalline cellulose. They were able to correlate dielectric frequency measurements with changes in the mechanism of moisture sorption, as the relative humidity was increased.

#### 1.4.4 Thermal analysis

Thermal methods of analysis can be used to determine water in pharmaceutical solids by the observation of bulk material effects such as sample weight or heat capacity. During thermal analysis, samples are typically subjected to linearly increasing temperature, and properties such as mass, evolved energy or evolved products, are monitored.

Thermogravimetric analysis (TGA) measures weight change against temperature, and is usually carried out in parallel with other thermal studies. Differential thermal analysis (DTA) and differential scanning calorimetry (DSC) give comparable information. DTA measures temperature difference  $\Delta T$  between a sample and reference, and DSC monitors the difference in energy supplied,  $d(\Delta q) / dt$ , to the sample and reference, in order to maintain a constant temperature ramp in the sample. Theories of DTA and DSC relate peak area to the heat of reaction, and peak shape to the mechanism of reaction: information on mass changes, enthalpy changes, activation energies and specific heats may be obtained (Wendlandt, 1974; Mackenzie, 1980; Mackenzie and Laye, 1986).

Applications of thermal analysis to solid dosage form technology are reviewed by Wesolowski (1985), and the use of DTA and DSC to determine binding enthalpies of water to biopolymers has been demonstrated by Zettlemoyer et al (1975). DTA has been applied to determine the fraction of bound water in starches, cellulose and foods by measuring the amount of water which is unfreezable at 0°C (Duckworth, 1971; Simatos et al, 1975).

Immersional calorimetry may be used to investigate solid-vapour interactions, since the thermodynamics of the immersion process and of vapour adsorption, are directly related (Zettlemoyer et al, 1975):

$$\Delta H_{ad}^P = \Delta H_{imm} - \Delta H_{imm}^P - N_{ad} \quad (21)$$

$\Delta H_{ad}^P$  = integral enthalpy of adsorption at vapour pressure P

$\Delta H_{imm}$  = enthalpy of immersion per unit area of solid

$\Delta H_{imm}^P$  = enthalpy of immersion for solid equilibrated with  
vapour pressure P

$N_{ad}$  = number of molecules adsorbed per unit area at  
vapour pressure P

This technique was utilised by Hollenbeck et al (1978) to study the interaction between microcrystalline cellulose and water. They were able to show that sorption hysteresis in this system is of both enthalpic and entropic origin. Enthalpies of adsorption for different grades of microcrystalline cellulose, were obtained using a microcalorimeter system by Sadeghnejad et al (1985).



A dual approach was adopted by Buckton and Beezer (1988) in the study of water vapour adsorption onto powdered drug surfaces. Enthalpies of adsorption were measured directly using a microcalorimeter system, and sorption data obtained using a vacuum microbalance were used to calculate the Gibb's function for adsorption. Combining these data allowed a value for the entropy of adsorption to be derived.

## 1.5 THE PROCESSING OF CONTAMINATED RAW MATERIALS INTO SOLID ORAL DOSAGE FORMS

The likelihood that micro-organisms contaminating dry raw materials will persist into the finished products, will depend upon the water activity to which the materials are subjected, and the process variables employed.

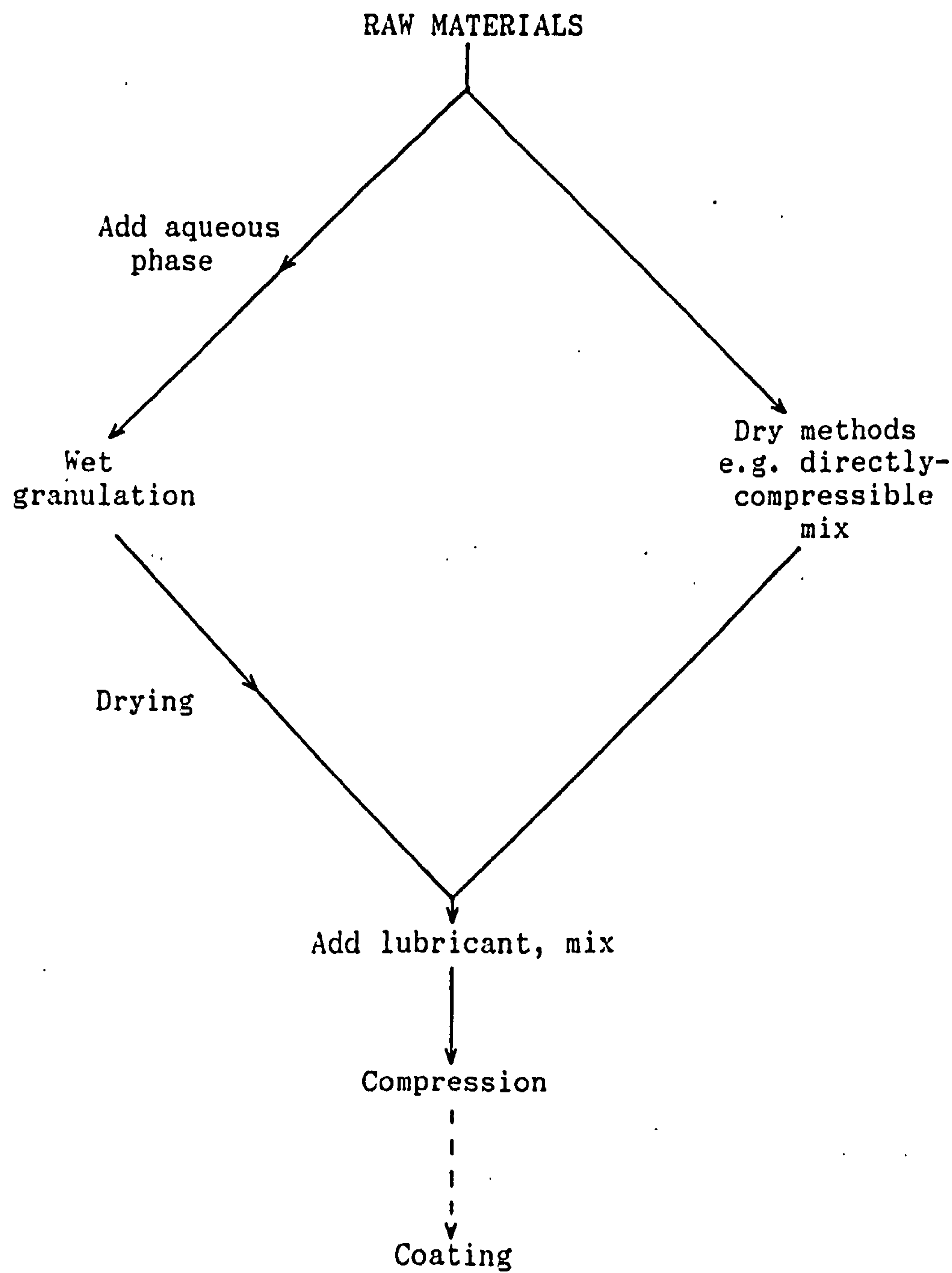
In their simplest form, hard gelatin capsules may be filled with powders which have been simply dry mixed, if necessary with an inert diluent powder to increase bulk.

From a microbiological standpoint, the tableting process may take one of two general routes (see figure 1.6): wet granulation involves the introduction of an aqueous phase, and a subsequent drying step; other methods, such as dry granulation, or direct compression, do not. Both capsule formulations and tablets may be produced from free-flowing, spherical granules (spheroids). The manufacture of spheroids involves first extruding a wet powder mass, then spheronising the extrudate and finally drying the granules. The survival of contaminating micro-organisms during extrusion and spheronisation has not been previously studied.

### 1.5.1 Wet granulation

During wet granulation, drug and excipients are formed into free-flowing and compressible agglomerates. The wet phase is usually water with a binding agent, such as a natural gum. Granulation techniques are reviewed by Barlow (1968) and by Cooper and Rees (1972). Carstensen et al (1976) examined the kinetic aspects of

Figure 1.6 A schematic representation of the stages of tablet processing





water distribution during wet granulation. They found that the process involves firstly the production of an "overwetted" granule, which equilibrates on further mixing.

Three stages of moisture distribution have been proposed as liquid is added during wet granulation (Rumpf, 1958; Newitt and Conway-Jones, 1958):

(i) water is held by capillary forces at the points of particle-particle contact (pendular stage);

(ii) the liquid film starts to envelop particles, internal voids are filled (funicular stage);

(iii) particle becomes surrounded by a liquid film, inter-particle friction is reduced and close packing takes place. Air in void spaces is displaced, and a concentrated suspension is formed (capillary stage).

Powder moistening may be accompanied by the partial dissolution of one or more components of the formulation, lowering the  $A_w$  of the particle suspension. The physical and chemical effects of moisture in tableting are reviewed by Griffiths (1969).

#### 1.5.2 Granule drying and the heat resistance of micro-organisms

The usual drying temperature for wet granulations is about 50°C, but may be lower for thermolabile substances. Forced convection ovens are used to dry granules spread on trays, and large batches may require up to 24 hours to dry (Rawlins, 1977). In a study by Kallings et al (1966) it was found that drying granules at raised

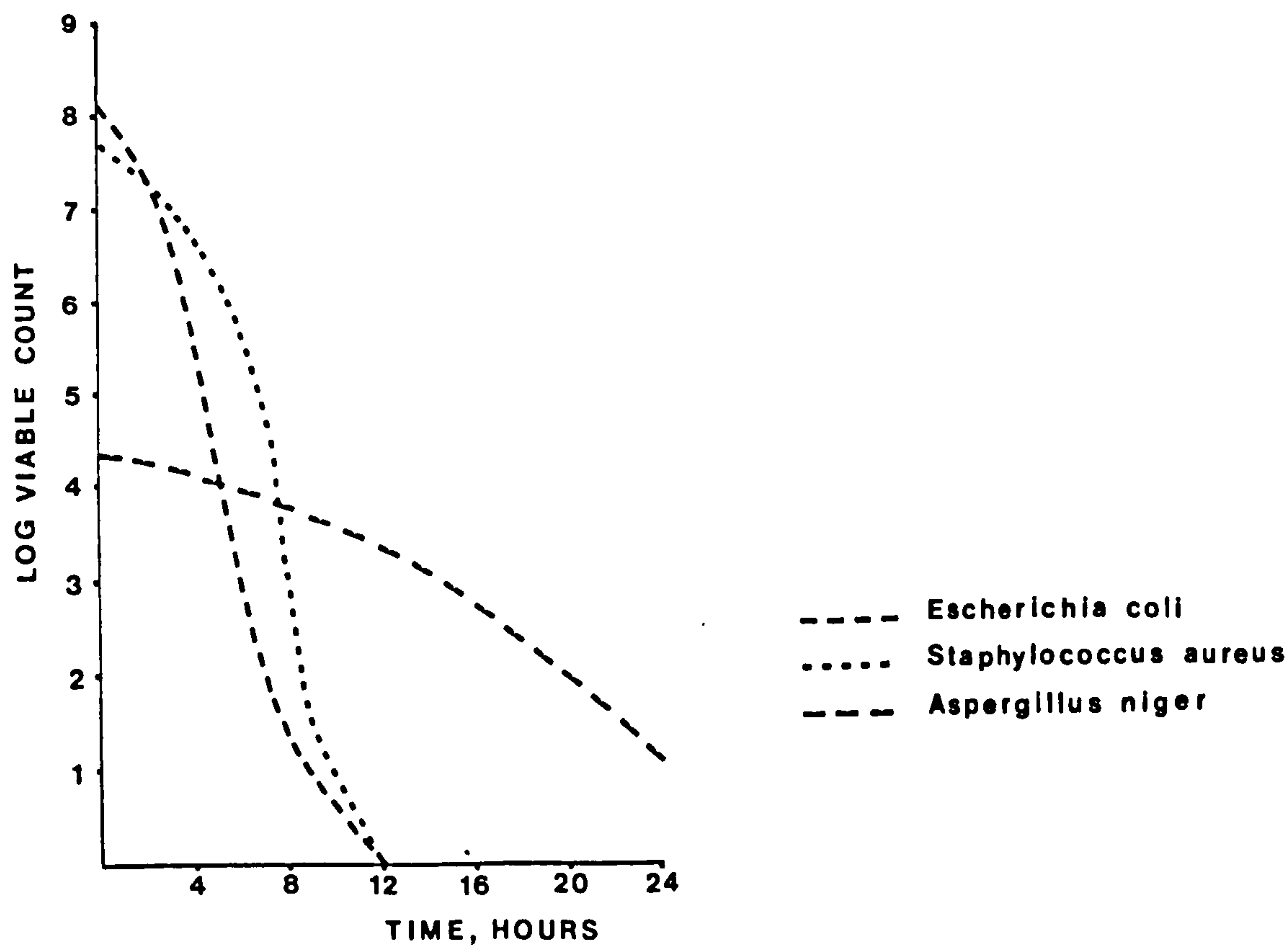
temperatures provided "excellent conditions for bacterial growth". However, no experimental data were given to support this observation. To investigate further, Fassihi and Parker (1977) subjected contaminated lactose and acacia granules to low temperature (40-45°C) drying. As can be seen from figure 1.7, the vegetative bacteria were all killed within 12 hours, and 99% of mould spores were killed in 24 hours.

The heat resistance of bacteria is influenced by a number of factors, including the drying temperature and time, and the substrate conditions (Hansen and Riemann, 1963). It is well known that the heat resistance of micro-organisms decreases with increasing humidity, and for this reason, "wet heat" is a more efficient sterilizing agent than "dry heat". Hansen and Riemann (1963) explain that when wet proteins are heated, peptide chains are freed by the loss of their water of hydration. Interpeptide bonds are formed instead, but only in the presence of water. However, in dry conditions, the number of water dipoles acting on the peptide chains is less. Protein dipoles are more important, and act to stabilize the peptide chains, so that more energy is required to free the peptides. This increases heat resistance overall. The kinetics of thermal inactivation of micro-organisms are detailed by Joslyn (1977).

Calhoun and Frazier (1966) studied the effect of available water on the heat resistance of some non-spore forming bacteria. They found that "protection" from the effects of heating depended on the organism, the  $A_w$  and the solute used to lower  $A_w$ . The

Figure 1.7 The effect of granule drying on the viability of bacterial cells and mould spores

(after Parker, 1984).





protective effect was enhanced for organisms previously grown in suspensions with lowered  $A_w$ .

Murrell and Scott (1966) studied the heat resistance of bacterial spores maintained at various  $A_w$  conditions during heating. They found that spores are more resistant to thermal inactivation when they are almost, but not completely, dry. The optimum  $A_w$  for heat survival was between 0.20 and 0.40 $A_w$ , depending on the species. As the values approached 0.00 $A_w$ , all species of spore showed increased sensitivity to heating.

Bullock and Lightbrown (1947), considered the spray-drying of micro-organisms. They observed that raised temperatures did not significantly enhance the level of kill achieved, and concluded that the destruction of organisms was due to the rapid removal of water from the cells. However, Loncin et al (1968) pointed out that organisms are protected from high temperature if the droplet size is large, and dehydration is therefore slow. The droplet temperature starts to rise more sharply when the moisture content reaches a low level, by which point the contaminating organisms are more resistant to heat.

### 1.5.3 Tablet compression

The increased use of directly-compressible tablet formulations gives microbial contaminants a greater chance of surviving the primary stages of tableting, since the lethal drying phase is avoided. As a result, the compaction process and its effect on micro-organisms have been examined. Significant factors in

determining the fate of organisms during compaction would appear to be the compaction pressure, speed, the nature of the formulation, and the distribution of the micro-organisms within the formulation.

#### 1.5.3.1 The mechanisms involved in powder compaction

There have been a number of theories put forward to account for microbial kill on tablet compression, and to explain the merits of each, it is necessary first to understand the mechanisms involved in powder compaction.

The process of powder compaction may be followed by monitoring the changing relationship between punch force and punch displacement. The measurement of applied force is achieved by instrumentation of the tablet press. The measuring systems used can be divided into two types: strain gauges and piezoelectric transducers. Strain gauges are pieces of metal foil which may be mounted in convenient positions on the press. An electric current runs through the foil, and applying stress to the foil results in a measurable change in resistance. A piezoelectric transducer is made of quartz and emits an electric charge, proportional to the force applied, when stressed. Applications of tablet press instrumentation are reviewed by Hoblitzell and Rhodes (1985).

Force-displacement curves aid the characterization of certain aspects of the compression process. For example, they have been used to characterize the tableting process both qualitatively and quantitatively, and to calculate the net work of compression (de

Blaey and Polderman, 1970; Ragnarsson and Sjogren, 1985) and the work to overcome die wall friction (de Blaey, 1972). For materials which are prone to capping, the data from force-displacement curves may be used to define optimal formulation and compression conditions (de Blaey et al, 1971). The interpretation of powder compaction data was reviewed by Krycer et al (1982).

Tablet formation by compression occurs through a number of simultaneous processes which bring about particle consolidation and bonding. Particle rearrangement, fracture and plastic deformation all act to increase the areas of interparticle contact (Hiestand et al, 1977).

During the tableting process there is unequal redistribution of powder within the die. The core of powder in the centre of the die moves relatively more than powder in contact with the die wall, resulting in uneven force distribution and areas of different compact density. The reason for this is thought to be that the interparticulate frictional forces are less than the particle-die wall frictional forces (Train, 1956). This effect of die wall friction may be overcome by the addition of a material with a low shear strength, such as magnesium stearate, which on mixing, forms a lubricating film around the other particles. However, lubricants are often hydrophobic in nature, and they can seriously reduce the wettability of the finished tablet, as well as its strength (de Blaey, 1972).

The type of punch used in compaction affects the compression



forces experienced by the powders. When flat-faced punches are used, forces act towards the bottom half of the compact, making this area relatively more dense. However, with curved punches forces act towards the centre of the powder compact, and the greater the angle of punch curvature, the closer to the punch face is the result of the force (Sixsmith and McCluskey, 1981).

A transmitted force has two components: a normal stress and a stress tangential to the normal stress, which is due to shearing. The normal stress is associated with volume changes, whereas the shear stress causes, or responds to, deformation. Axial pressure applied through the upper punch is transmitted as a shearing force. Force is also transmitted radially to the die wall, and this radial pressure depends upon the elasticity or plasticity of the material (Leigh et al, 1967).

The formation of a satisfactory tablet depends on the ratio of stress relaxation to elastic recovery of the powder system. The stress relaxation reflects the plasticity of a compressed system, causing the applied force to decay with time. The elastic recovery describes the elastic energy stored during compression, which on decompression may be sufficient to break interparticulate bonds and result in a fragile tablet. Fracture may occur in a powder system when the stresses within the particles reach a magnitude sufficient to cause crack propagation (Hiestand et al, 1977).

Heckel (1961) described the relationship between compact density

and applied pressure (equation 22):

$$\ln (1 / 1 - D ) = C_p + I \quad (22)$$

Where D is the relative density, equivalent to the bulk density at pressure p, divided by the true (calculated) density. C is the material constant, equal to the reciprocal of the mean yield pressure of the material, and I relates to particle movement during the initial stages of compaction.

It has been shown that the Heckel equation can be used to distinguish between powder systems which plastically deform, and those which fracture, on compression (Hersey et al, 1973; Jones, 1977). A plot of  $\ln(1 / 1 - D)$  versus p will tend towards a straight line for materials such as sodium chloride which show plastic deformation characteristics, whereas for fracturing materials such as lactose, the line is curved at the lower values of p.

During powder compression, energy in the form of heat, is generated. It has been suggested that localized melting at the points of interparticulate contact may aid bonding for materials with relatively low melting points (York and Pilpel, 1973). They showed that for tablets prepared from a series of fatty acids, the tensile strength decreased as the melting point increased. It has been observed that the increase in tablet temperature is directly related to speed of compression and the magnitude of the applied pressure (Hanus and King, 1968; Travers and Merriman, 1970).

Compression speed has its own effects on the properties of

tablets. Increasing compression speed was found to result in harder tablets, and this effect was enhanced at higher compaction pressures (Fassihi et al, 1980). The time-dependent nature of plastic flow was demonstrated by increasing the punch velocity (Roberts and Rowe, 1985). It was shown that for starch and polymeric materials, increasing the punch speed resulted in an increased yield pressure. This was thought to be due to either a change to more brittle behaviour or a reduction in plasticity. The tendency to exhibit brittle behaviour and to resist deformation, was found to be increased for particles milled to a smaller size (Roberts and Rowe, 1986).

#### 1.5.3.2 Microbial kill on tablet compression

Microbial contaminants of tablet powders, may experience potentially damaging stresses during the compaction process, and the nature and magnitude of these stresses will vary with processing conditions and the formulation used.

Early investigations were carried out by Chesworth et al (1977), who studied the compression of natural raw materials, and the effects on their microbial content. They observed about a 70% drop in total viable count on compaction, and found that the temperature of tablets rose to between 40 and 50°C. The lethal effect was thus attributed to heating and mechanical damage by shearing forces.

Fassihi et al (1977b) observed a linear relationship between log per cent survival and applied pressure for Aspergillus niger



spores, incorporated with a tablet formulation via the granulating fluid. They derived a relation (equation 23) to characterize death rate varying with applied pressure:

$$N_p = N_o e^{-dp} \quad (23)$$

Where  $p$  = applied pressure

$N_o$  = initial number of spores

$N_p$  = number of spores surviving at applied pressure  $p$

$d$  = death rate constant.

These workers proposed that at low compaction pressures, kill was due to mechanical disruption. At higher pressures, they proposed that kill was effected by high local surface temperature, due to the formation of "localized hot spots" at the points of interparticle contact.

Conversely, Yanagita et al (1978) observed a non-linear relationship between pressure and survival. They found that larger cells were more likely to be killed than smaller cells, and thus considered shearing to be the major lethal agent. However, the possible contribution towards microbial kill made by raised temperature was also acknowledged.

Plumpton et al (1982) argued that micro-organisms are killed during tablet compression by shearing forces, and that temperature increases are not a significant factor. They pointed out that although tablet temperatures may rise on compaction, temperature increases are at the most, no greater than 15-20°C (Hanus and King, 1968; Travers and Merriman, 1970), and are of short

duration.

Plumpton et al (1982) were able to demonstrate that the kill of yeast or mould spores in direct-compression materials depended on a number of factors, namely, compaction pressure, compaction speed, the ratio of organism size to excipient particle size, and the mechanism of compaction of the excipient. Increasing compaction speed was found to reduce the level of kill achieved, and this was thought to be due to stress relaxation at slower speeds, generating greater shear forces. Larger cells were more susceptible to kill on compression than smaller ones, which may be explained by the greater effect of shear on larger cells. For materials which fracture on compression, kill was proportional to applied pressure over the whole pressure range, whereas for more plastically-deforming materials, a decrease in the ratio of applied pressure to per cent kill was observed as minimum porosity was approached.

Further studies have indicated that microbial kill during tableting is dependent upon the compression behaviour of the materials used. The inactivation of yeast cells in a directly-compressible formulation was found to vary with cell size fraction (Plumpton et al, 1986a); once again, non-linearity between per cent kill and pressure was observed, using potassium chloride which has plastic deformation characteristics. Killing of Bacillus megaterium spores was correlated to the compaction mechanism of the excipients used by Plumpton et al (1986b). For bacterial cells and spores, Fassihi and Parker (1987) noted linear "pressure-

survival" plots for brittle materials, and non-linear plots for plastically-deforming materials. The degree of kill was greater where brittle materials were used.

The relative importance of shear forces in microbial inactivation is still the subject of debate. The effects of variables such as excipient size, organism size and distribution, were attributed to differential shearing by Plumpton et al (1982, 1986a, 1986b). However, Fassihi and Parker (1987) argue that the situation is more complex, and that both compression force and compression speed have a direct killing effect. This lethal effect will vary according to other factors, such as the effective area of interparticle contact and the tendency towards fracture, as well as the thermal conductivity, specific heat and density of the other materials.

#### 1.5.4 Extrusion/spheronisation

One way of differentiating between shearing and compressional effects on powders is to study the extrusion of a wet powder mass, where some of the forces acting on the particles prior to and during extrusion have been previously characterized (Harrison, 1982).

The principle of wet mass extrusion is that a maleable, wet powder mass, with suitable rheological characteristics, is forced from a large diameter reservoir through one or more smaller diameter orifices (dies) under controlled conditions. The material undergoing extrusion is subject to compression forces, which



result at first in rearrangement to the apparent particle density, and then in powder flow. A large proportion of the pressure loss associated with extrusion involves flow from the reservoir into the narrower die entry. This was termed the upstream pressure loss by Harrison (1982).

The flow of the powder mass through the narrow die evokes shearing forces, and the shear stress acting upon material flowing through the die capillary is proportional to the distance from the centre of the capillary. Thus the shear stress at the die wall will depend upon the radius of the die, and can be described by the following relation:

$$\tau_w = \Delta p r / 2L \quad (24)$$

where  $\tau_w$  = shear stress at the die wall

$\Delta p$  = the pressure drop along the capillary

$r$  = the radius of the capillary

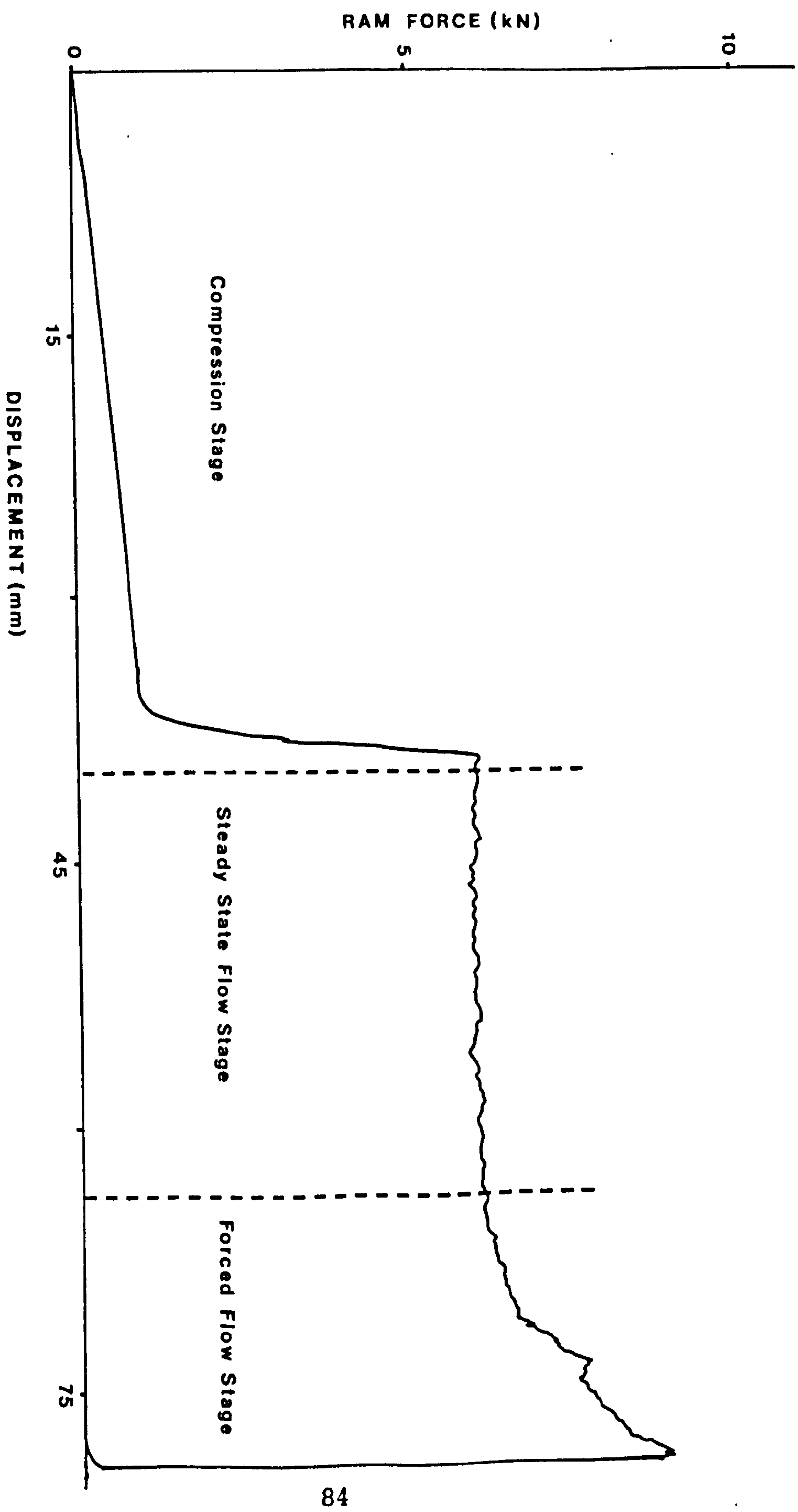
$L$  = the length of the capillary.

The extrusion process may be followed by plotting force-displacement curves of ram force against piston displacement (figure 1.8). The magnitude of shear at the die wall may be calculated from plots of ram pressure (the ram force divided by the area of the ram piston) against the length to radius ratio of the die. The slope of the curve divided by two, gives the mean shear stress at the die wall, with intercept equivalent to  $p_0$ , the upstream pressure loss.

Extrusion/spheronisation as used commercially, involves the use of

Figure 1.8 Force-displacement profile for ram extrusion  
of a wet powder mass

(after Harrison, 1982).



multi-orifice extruders. Increasing the number of dies has the effect of reducing the shear rate through the die. The constant diameter extrudate is fed onto the spheroniser, which consists of a circular friction plate with a series of radial and concentric grooves, which spins inside a stationary cylinder. It has been observed that the spheroniser plate cuts the extrudate into lengths equivalent to the die diameter (Conine and Hadley, 1970). The material is then rolled into spherical granules (spheroids), and spray-dried.



## 1.6 THE PRESERVATION OF SOLID ORAL DOSAGE FORMS

The dry nature of tablets and the effects of processing will suppress microbial growth. In addition, the use of specific antimicrobial treatments has been studied.

### 1.6.1 Ethylene oxide gas

Treatment with ethylene oxide gas is particularly suitable for solid products, since the gas diffuses through paper or plastic containers so that products can be sterilized in their final packaging. The gas exerts its antimicrobial effect by alkylating groups in enzymes, proteins and peptides. The efficiency of the sterilization treatment depends upon the time of exposure to the gas, the relative humidity and temperature used, the partial pressure of the gas, and the successful penetration of the gas into the material.

Ethylene oxide treatment was applied to crude drugs using samples containing natural populations of up to  $3 \times 10^6$  organisms per gram (Diding et al, 1968). It was found that where humidification of the material or penetration of the gas was incomplete, a sterilization effect was not attained. However, tablet raw materials, including acacia containing  $1.1 \times 10^5$  organisms per gram, were successfully sterilized using ethylene oxide for 12 hours at  $45^{\circ}\text{C}$  and 65% RH (Fassihi, 1978).

Graf et al (1985) compared different antimicrobial treatments applied to pancreatin containing up to  $1.2 \times 10^5$  organisms per gram. In this study, the aim was to find a method which would adequately

reduce the microbial load, without excessively reducing the enzyme activity. This was achieved by exposing the pancreatin to low concentration ethylene oxide gas for a short time interval. The authors note the disadvantages of routinely using ethylene oxide, in that its alkylating effect can damage human tissue and thus extreme care must be taken to ensure that gas residues do not remain in treated products.

#### 1.6.2 Gamma irradiation

Small doses of gamma radiation are harmful to micro-organisms. Cobalt-60 gamma radiation was applied to non-sterile packaged tablets and powders by Safarov et al (1983), who noted that the treatment reduced the microbial count, but that this was accompanied by colour changes in some materials. Despite the colour changes, no drug decomposition products could be detected; the drug content and other physicochemical properties were unchanged. Graf et al (1985) observed that pancreatin lipase was inactivated by gamma radiation in a dose-related manner, so that radiation treatment should only be applied to lightly contaminated products.

#### 1.6.3 Antimicrobial preservatives

The inclusion of antimicrobial preservatives into finished solid dosage forms has been investigated. In experiments carried out by Payne and Smart (unpublished), it was found that the inclusion of 0.1% w/w propyl p-hydroxybenzoate in Paracetamol Tablets B.P. slowed and inhibited the growth of moulds, under conditions of high humidity. For tablets prepared from lactose and potato



starch, the inclusion of 0.075% to 0.1% w/w parabens (methyl-, ethyl-, or propyl-p-hydroxybenzoic acid) was found to protect against subsequent fungal challenge (Fassihi et al, 1978). It was found that tablet granules which had been sprayed externally with an aqueous or alcoholic solution of preservative were better protected than granules into which preservative had been incorporated.

Aqueous based film coating procedures use solutions of cellulosic polymers which may readily support microbial growth (Banker et al, 1982). Where large batches of solution are prepared in advance, preservatives may be added to prevent subsequent microbial proliferation in pumps, lines and coating equipment. The results of Fassihi et al (1978) suggest that a preserved film coating could protect the finished tablets from possible microbial growth during storage.

In general, however, it is unlikely that the inclusion of antimicrobial preservatives to prevent growth in tablet formulations will become widespread. Microbial quality assurance for tablets is achieved by the use of suitably "clean" raw materials which comply with the specifications of regulatory bodies and manufacturers, and by the strict adherence to GMP during processing. Any justification of the need for a preservative might infer non-adherence to GMP. In addition, there are only a limited number of preservatives suitable for oral use: mostly weak acids and their derivatives, showing pH-dependent activity. There is a wealth of literature concerning the



interactions between preservatives and pharmaceutical solid raw materials or container materials (e.g. Bean, 1972; Clarke and Armstrong, 1972; Coates, 1973; Yousef et al, 1973). It is therefore likely that for solids at least, assurance of effective preservation lies in the screening of raw materials, the reduction of microbial load during processing, and the understanding and control of the water-sorbing tendencies of the finished product.

## AIMS

The purpose of this work was to study some factors affecting the survival of vegetative bacteria, present as contaminants of tablet excipients which are directly-compressible. This was achieved by using a spoilage isolate, Enterobacter cloacae, which is a Gram-negative, free living water-borne organism, and for comparison, a standard strain of Staphylococcus aureus, which is a Gram-positive, pathogenic species.

1. Contaminated, directly-compressible excipients were prepared for tableting studies.
2. Changes in the viability of the contaminating bacteria during tableting, extrusion and spheronisation was measured, and consideration given to the forces acting upon the bacteria during the compression process.
3. The influence of water availability on the survival of bacteria during storage in solid products such as tablets was investigated, in an attempt to understand the conditions under which non-spore forming organisms are able to survive in "dry" pharmaceutical products.

Although this thesis is primarily concerned with tableting, the results may affect considerations given to the processing and storage of other types of non-sterile formulations, where reduced  $A_w$  is the means of preservation.

## 2. MICROBIOLOGICAL METHODS AND MATERIALS



## 2.1 MEDIA FOR ROUTINE USE

### 2.1.1 Tryptone soya broth

Tryptone soya broth (Oxoid, code CM129) was prepared by adding 30g of broth powder to one litre of distilled water. The suspension was mixed and distributed in 9ml aliquots into 20ml glass screw-capped MacConkey bottles. Broths were sterilized by autoclaving at 121°C for 15 minutes.

### 2.1.2 Nutrient broth

Nutrient broth (Oxoid, code CM2), prepared by mixing 13g of powder with one litre of distilled water, was distributed and sterilized as above.

### 2.1.3 Tryptone soya agar

Tryptone soya agar was used for all bacterial counts, unless stated otherwise. It was prepared by suspending 20g tryptone soya agar powder (Oxoid, code CM132) in 500ml of distilled water, and sterilized as above.

### 2.1.4 Agar plates

20ml of sterile molten tryptone soya agar was poured aseptically into sterile disposable petri dishes, and allowed to set. The plates were overdried at 37°C for 72 hours, or at room temperature for one week.

## 2.2 DILUENTS

### 2.2.1 Peptone buffer

A phosphate buffer solution, containing 0.1% peptone, was prepared according to the formula below (Buhlmann, 1968):

$\text{KH}_2\text{PO}_4$	3.56g
$\text{Na}_2\text{HPO}_4 \cdot \text{H}_2\text{O}$	7.23g
NaCl	4.3g
peptone	1.0g
distilled water to	1000ml (pH 7.0)

The solution was distributed in 9ml aliquots into 20ml metal-capped boiling tubes, or in 99ml aliquots into 100ml glass screw-capped bottles, and autoclaved at 121°C for 15 minutes to sterilize.

### 2.2.2 Distilled water

Distilled water, from an all-glass still, was sterilized by autoclaving at 121°C for 15 minutes.

## 2.3 VIALE COUNTING PROCEDURES

### 2.3.1 Samples for viable counts

To determine the viable counts of solids (powders or tablets), semisolids (wet solids or suspensions of powders) or bacterial suspensions, a minimum of two samples were accurately weighed (1g) or measured (1ml) as appropriate, into 9ml diluent. Solid samples were disintegrated or dissolved, and the suspensions dispersed

with the aid of an electrical mixer (Gallenkamp Spinmix). The diluent used for all viable counts was peptone buffer, prepared as described in section 2.2.1 above, unless specified otherwise. The suspensions of 1g or 1ml sample with 9ml diluent, were serially diluted by transferring 1ml into 9ml or 99ml aliquots of diluent, and plated out immediately.

### 2.3.2 Miles and Misra technique

The surface viable counting method of Miles and Misra (1938) was used where the solid suspensions for plating were suitably dilute, and the anticipated viable count was sufficiently high ( $> 2 \times 10^3/\text{ml}$ ). Sterile glass dropping pipettes (Hoslab Ltd.) were fitted with 25SWG gauge needles and clamped vertically in a laminar flow cabinet. When filled with the bacterial suspension, the pipettes delivered a mean volume of 0.018ml per drop (see table 2.1) at a constant delivery rate of one drop per second. Ten drops were delivered onto overdried tryptone soya agar plates (section 2.1.4), the liquid was allowed to soak in before the plates were inverted and incubated at  $37^\circ\text{C}$  for 18-24 hours.

### 2.3.3 Spread plates

Spread plates were used where the numbers of viable bacteria in the samples was expected to be small, so that the solid concentration in the suspensions for plating was too high to use dropping pipettes. Overdried plates were inoculated with 0.5ml or 1ml of suspension, appropriately diluted, which was spread over the surface of the plates using a sterile, bent glass rod. The



Table 2.1 Volume delivered by dropping pipettes

Weight (g) of 5 drops of water delivered by each of 20 dropping pipettes			
0.0879	0.0897	0.0911	0.0902
0.0903	0.0890	0.0900	0.0890
0.0908	0.0907	0.0903	0.0908
0.0915	0.0879	0.0906	0.0888
0.0896	0.0892	0.0896	0.0893

Mean weight of 5 drops = 0.0898g

Range = 0.0879 - 0.0915g

Maximum deviation  
from the mean = 0.0019g = 2.13%

Standard deviation = 0.0010g = 1.11%

Mean weight of 1 drop = 0.018g

liquid was allowed to dry in before the plates were inverted and incubated at 37°C for 18-24 hours.

#### 2.3.4 Viable count results

Duplicate plates were prepared from each dilution (as described in sections 2.3.2 and 2.3.3 above) and the average count recorded. For each equivalent set of samples, the sample mean,  $\bar{X}$ , the sample standard deviation,  $\sigma_{n-1}$ , and the per cent coefficient of variation, % cv, were calculated. The statistical significance of variances in results within and between sample batches, was analysed by a t-test method appropriate for small samples (Bailey, 1981). The confidence interval for significance was 95 per cent (P less than or equal to 0.05).

### 2.4 TABLET EXCIPIENTS

#### 2.4.1 Excipients selected for study

Maize starch, Avicel PH101 (microcrystalline cellulose) and lactose were selected for these investigations on the basis of their common use as excipients in solid oral dosage forms, and their different relative hygroscopicities and nutritional qualities which may affect bacterial growth. The characteristics of maize starch were compared with two other starches, wheat starch and potato starch. Some grades of microcrystalline cellulose with sodium carboxymethylcellulose (sodium CMC) were included in the studies (Avicel RC581, Avicel RC591, Avicel CL611), for comparisons with results from work with Avicel PH101. For experiments where lactose was included, lactose 170 mesh was

used, except for the investigations of extrusion/spheronisation where the lactose was 300 mesh, in accordance with an established formulation. Magnesium stearate was used as a lubricant in tableting studies. Some of the excipient powders were gold-coated and photographed by scanning electron microscopy. Plates 1 and 2 show the different morphologies of the powders examined. Table 2.2 gives a full list of the excipients used.

#### 2.4.2 Total viable counts of excipients

##### 2.4.2.1 Method to determine the numbers of bacteria

A 10g sample of each excipient, as supplied, was mixed with 90ml peptone buffer solution, containing 0.1% Tween 80. Triplicate 1ml samples were withdrawn and serially diluted in 2 sets of peptone buffer (9ml). The suspensions were plated out onto tryptone soya agar spread plates, as described above in section 2.3.3. The tryptone soya agar plates were incubated at 37°C and examined for bacterial growth after 18-24 and 48 hours.

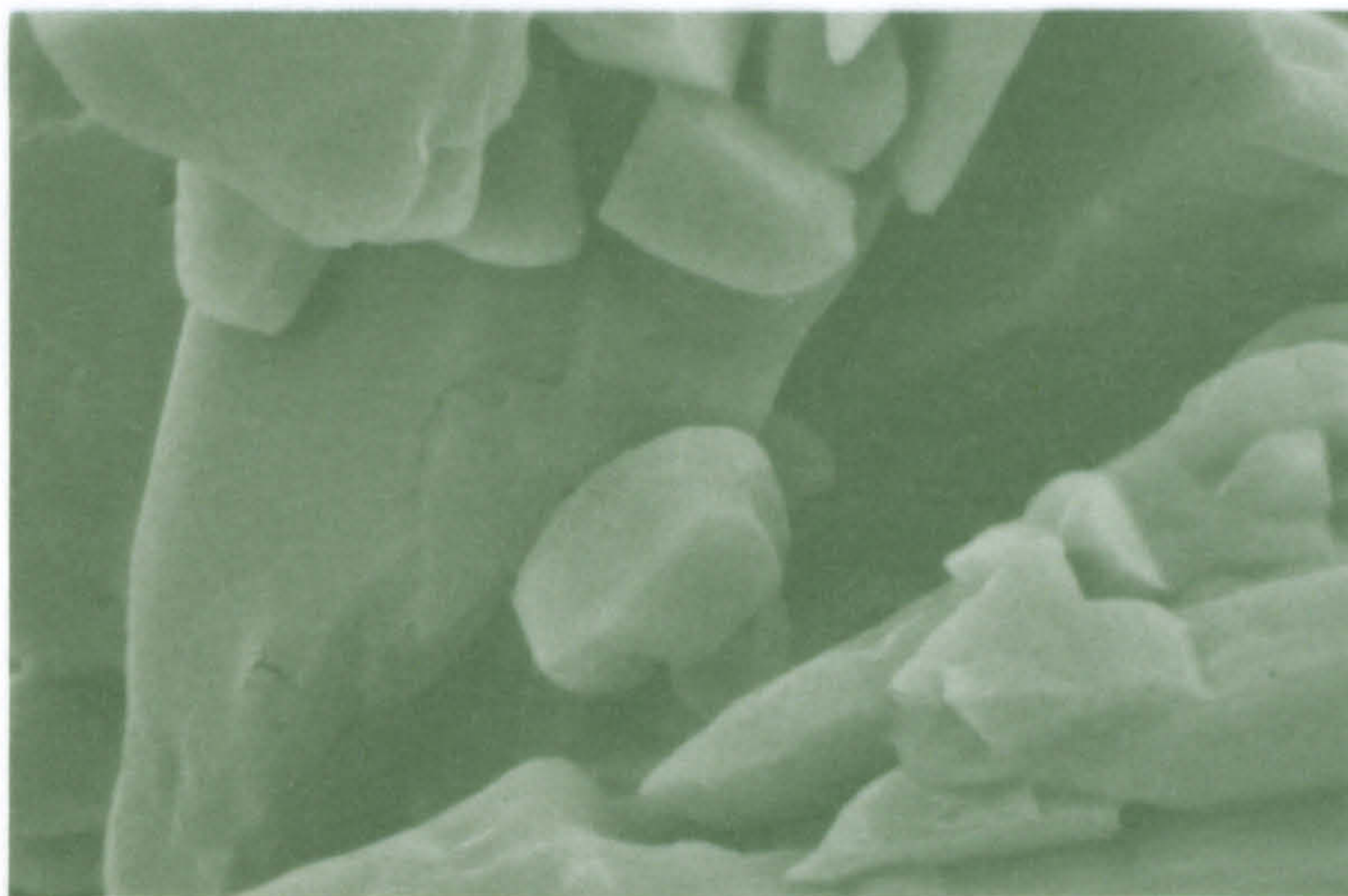
##### 2.4.2.2 Method to determine the numbers of fungi

Samples of the excipients were diluted in peptone buffer as described above (2.4.2.1), and plated out onto spread plates prepared from Sabouraud dextrose agar plates (Oxoid code CM41), according to the method described above in sections 2.3.3. The Sabouraud dextrose plates were incubated at 30°C, and examined for signs of fungal growth at intervals between 18 hours and five days.

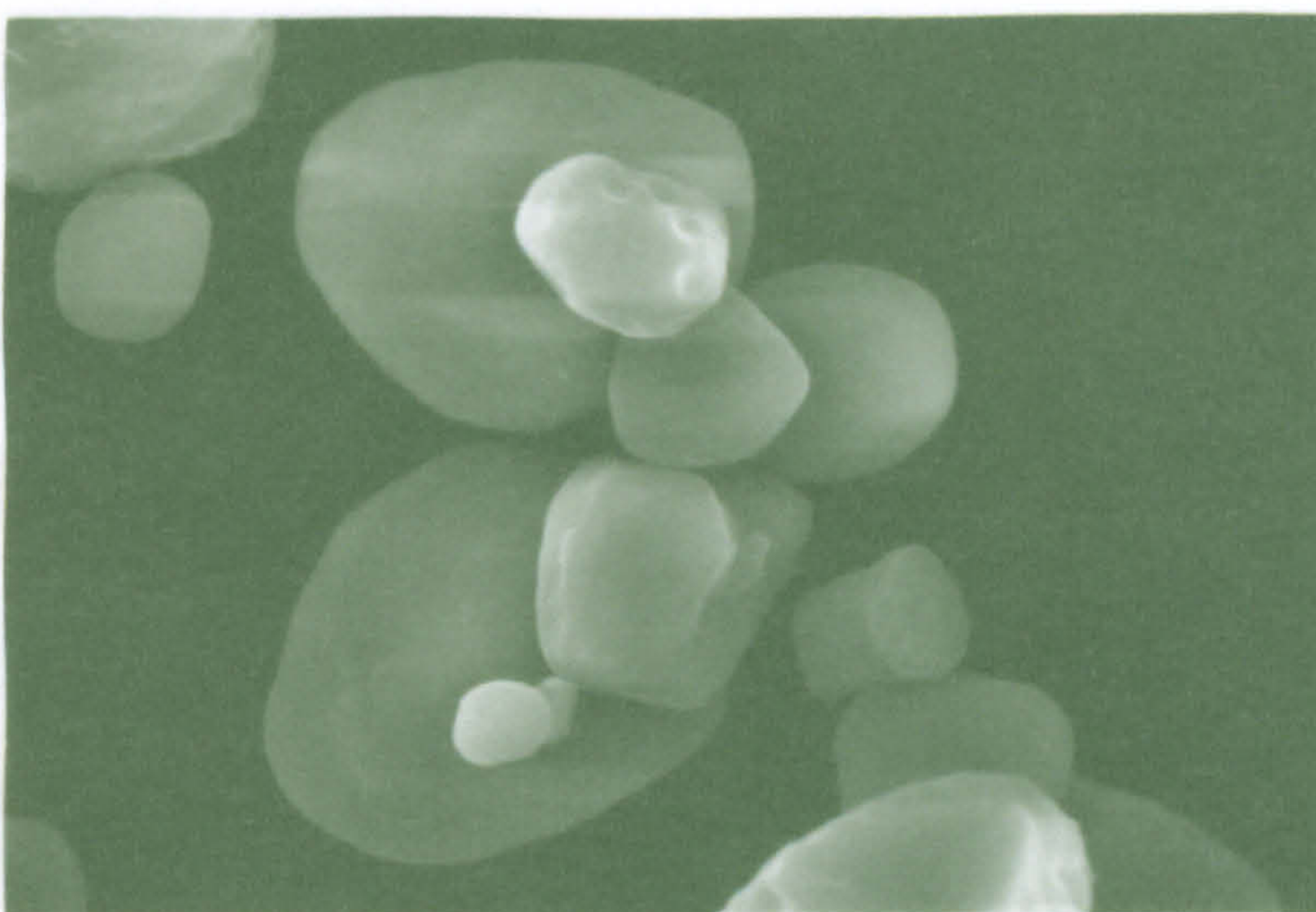


Plate 1. Scanning electron micrographs of tablet excipients

(a) Lactose 170 mesh (x880)



(b) Maize starch (x440)



(c) Avicel PH101 (x6900)

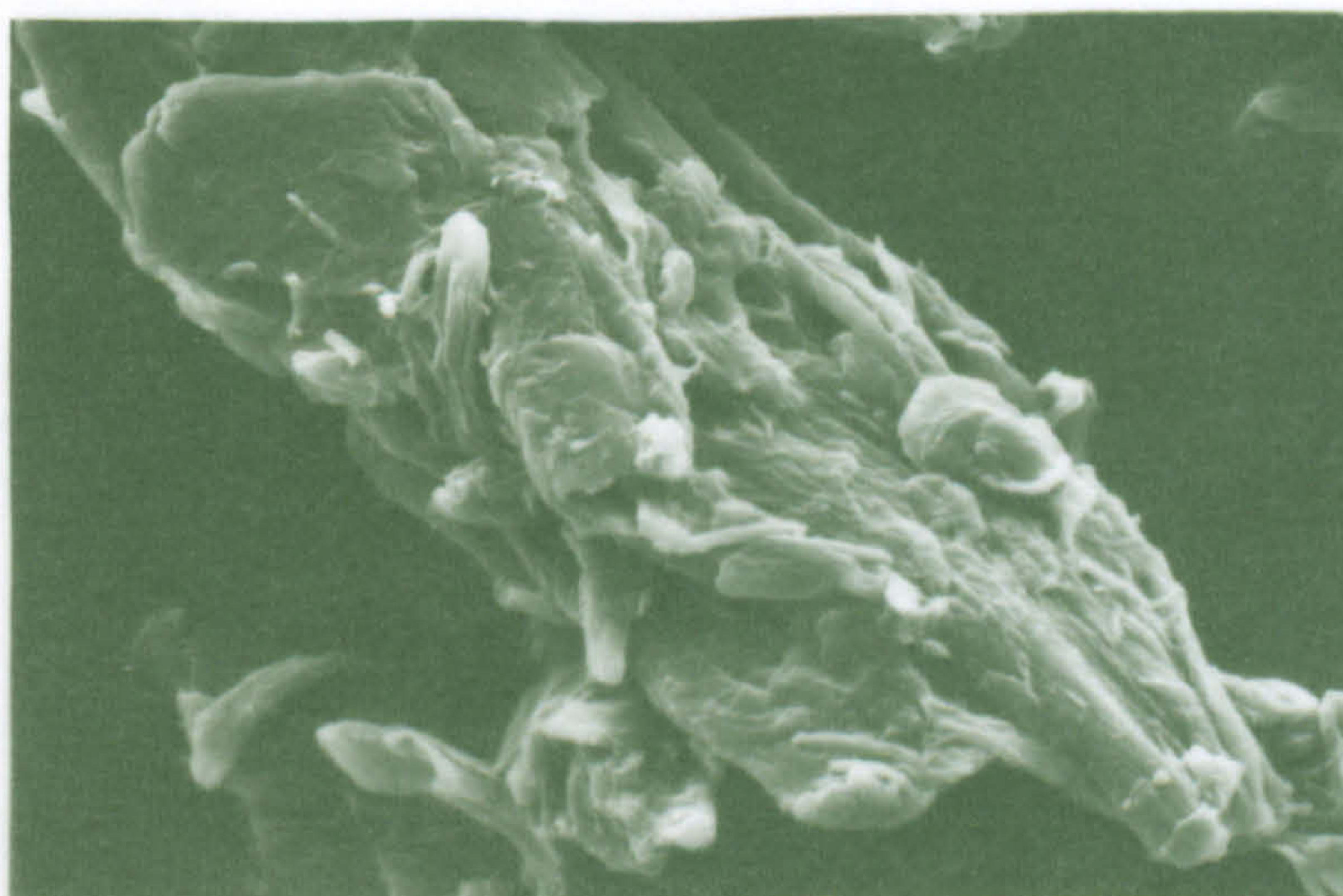
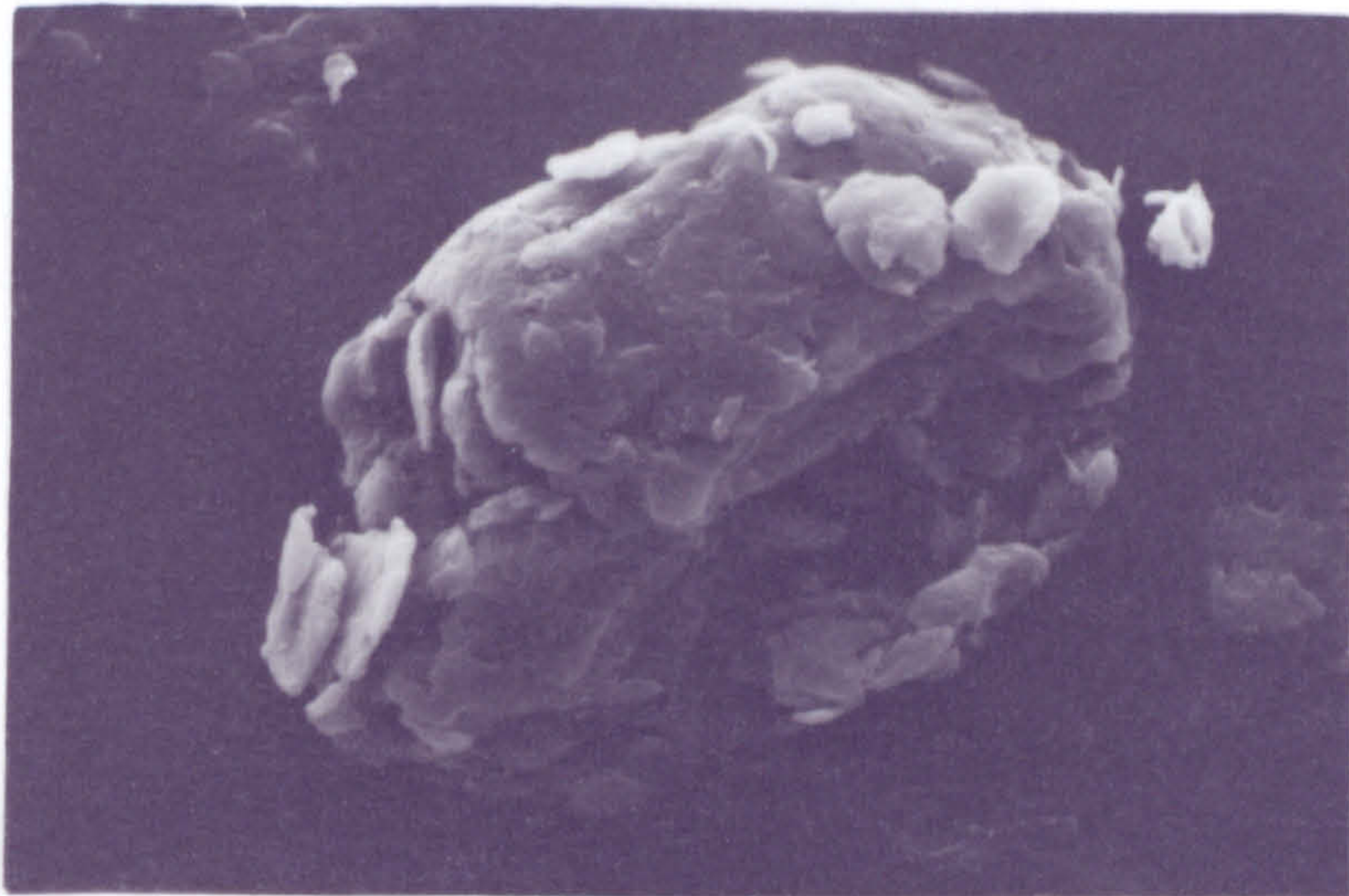


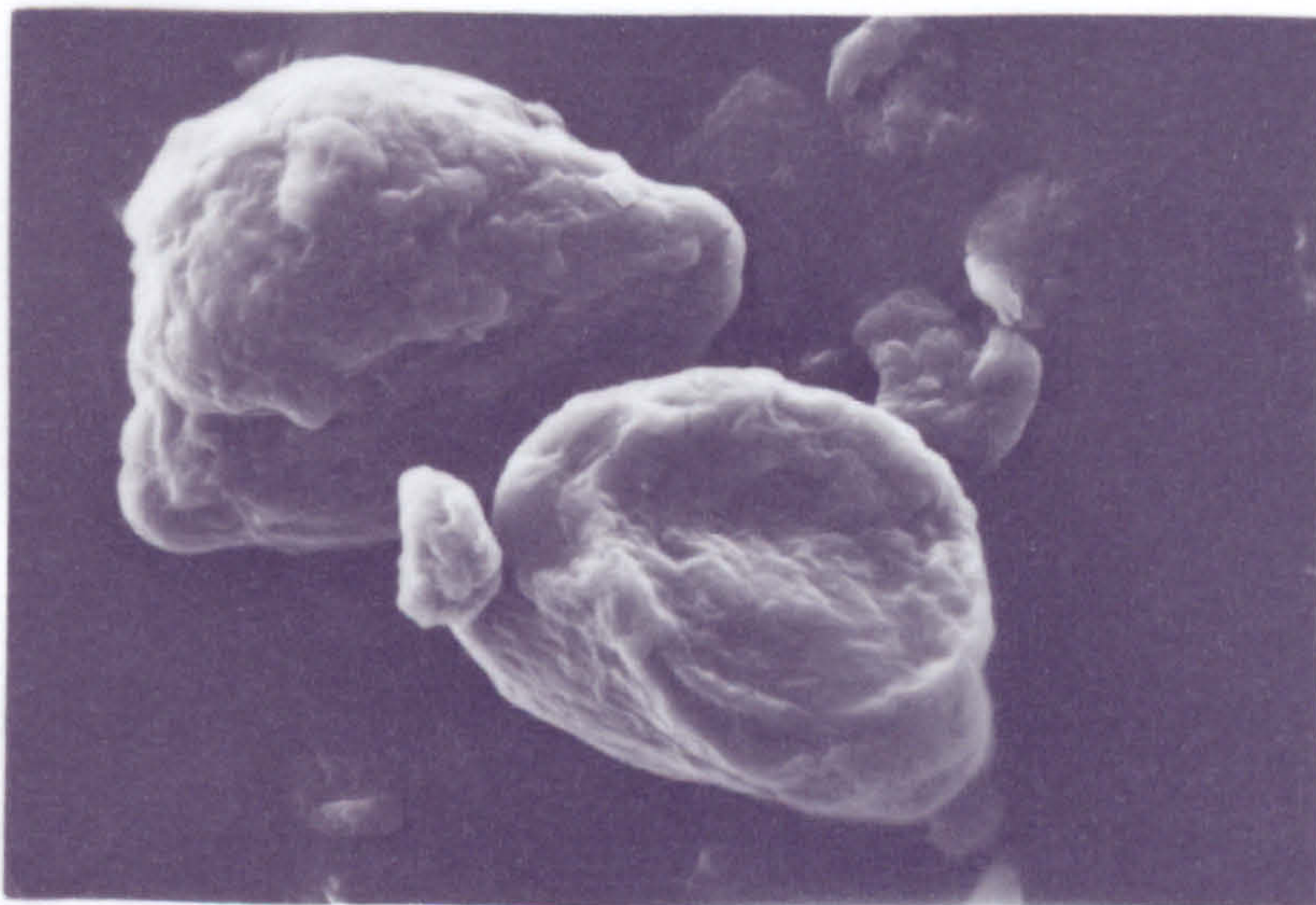


Plate 2. Scanning electron micrographs of three grades of Avicel

(a) Avicel RC581 (x880)



(b) Avicel RC591 (x880)



(c) Avicel CL611, surface detail (x6900)

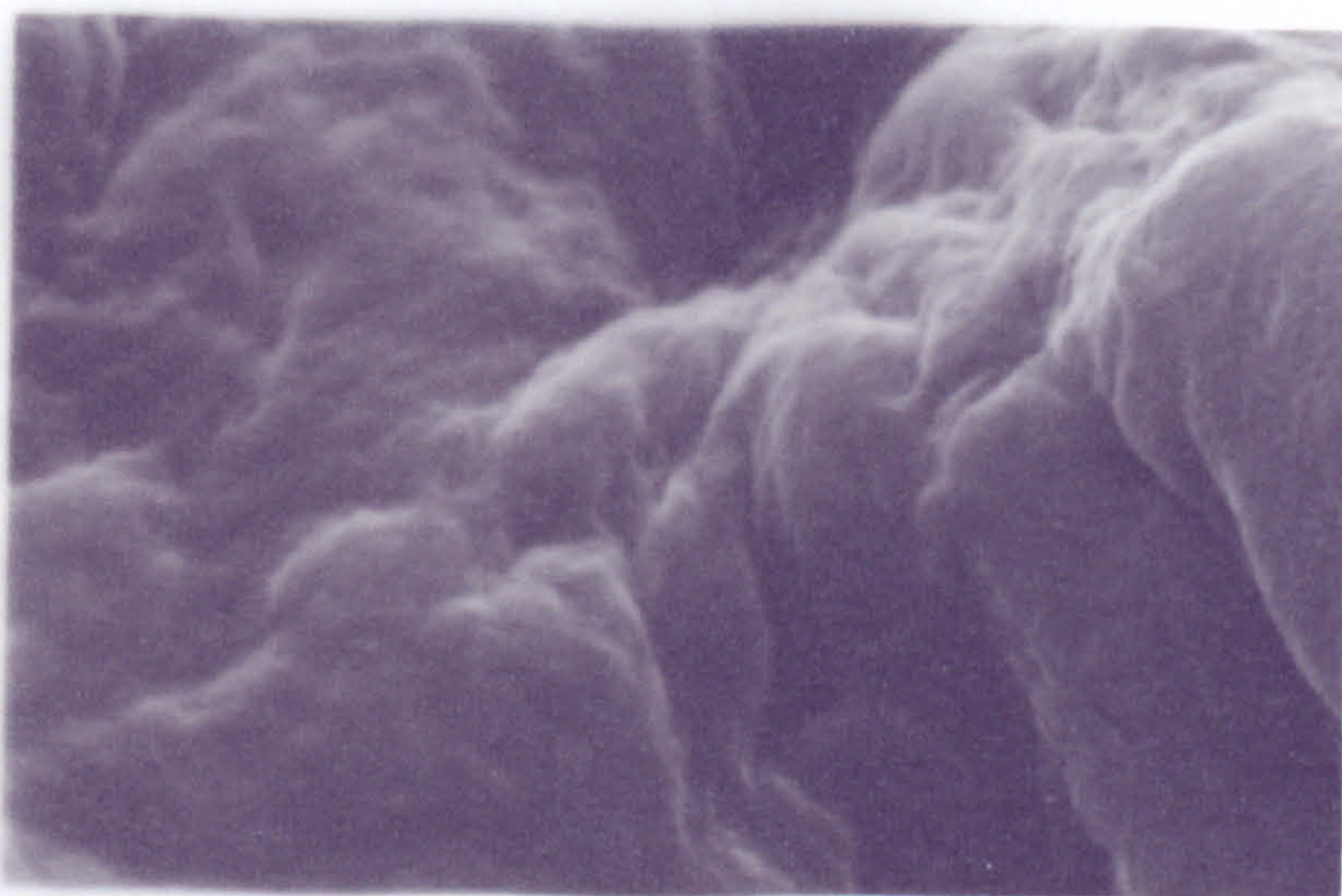




Table 2.2 Tablet excipients

Excipient	Supplier	Batch No.
Maize starch B.P., special	Roquette (U.K.) Ltd.	WD2072 *
Potato starch	BDH Ltd.	4927880H
Wheat starch	BDH Ltd.	5089980H
Lactose B.P., 300 mesh +	Dairy Crest	00130
Lactose B.P., 170 mesh	Dairy Crest	WC9442 *
Avicel PH101	Honeywill and Stein Ltd.	1629
Avicel CL611	Honeywill and Stein Ltd.	E8033
Avicel RC581	Honeywill and Stein Ltd.	ref.F237
Avicel RC591NF	Honeywill and Stein Ltd.	D7421
Magnesium stearate B.P.	Durham Chemicals Distributors Ltd.	WC4333 *

\* Excipients supplied by Glaxo Group Research Ltd.

+ Used for extrusion and spheronisation studies only.



Table 2.3 Total viable counts of tablet excipients

Excipient	Total viable count (cfu/g)	
	Bacteria	Fungi
Maize starch B.P., special	36	10
Potato starch	148	28
Wheat starch	119	37
Lactose B.P., 300 mesh	< 10	< 10
Lactose B.P., 170 mesh	< 10	< 10
Avicel PH101	< 10	23
Avicel CL611	< 10	13
Avicel RC581	< 10	< 10
Avicel RC591NF	< 10	< 10
Magnesium stearate B.P.	58	16

#### 2.4.2.3 Results of total viable counts

Table 2.3 shows the total viable counts of the tablet excipients.

#### 2.4.3 Tests for specified micro-organisms

Cultures obtained from total viable counts were tested for the presence of the pathogenic bacteria specified in the British Pharmacopoeia (1988). Tryptone soya broth was used as the pre-enrichment media, incubated at 37°C for 18 hours.

##### 2.4.3.1 Escherichia coli

1ml of the pre-enrichment media from each excipient culture was inoculated into 9ml MacConkey broth (Oxoid code CM5a), and incubated at 37°C for 24 hours.

Result: No isolate produced acid or gas in MacConkey broth.

Conclusion: E. coli negative.

Control: 1ml of a 1:10<sup>7</sup> dilution of a 24 hour E. coli broth culture was incubated with 9ml MacConkey broth. Both acid and gas were produced in the MacConkey broth. The broth was streaked onto MacConkey agar giving pink/red colonies after 24 hours at 37°C.

##### 2.4.3.2 Pseudomonas aeruginosa

Each of the pre-enrichment broths was streaked onto cetrimide agar, consisting of nutrient agar (Oxoid code CM3) prepared with 0.3% w/v cetrimide powder) and incubated at 37°C for 48 hours.

Result: No growth on cetrimide agar.

Conclusion: Ps. aeruginosa negative.

Control: A Ps. aeruginosa culture in tryptone soya broth was streaked onto cetrimide agar, and showed positive growth after incubation at 37°C for 48 hours.

#### 2.4.3.3 Staphylococcus aureus

Each of the pre-enrichment broths was streaked onto Baird-Parker agar (Oxoid code CM275) and incubated at 37°C for 24 hours.

Result: No growth on Baird-Parker agar.

Conclusion: Staph. aureus negative.

Control: A Staph. aureus culture in tryptone soya broth, was streaked onto Baird-Parker agar. Black, shiny colonies were produced after incubation at 37°C for 24 hours.

#### 2.4.3.4 Salmonella

Each of the pre-enrichment broths was streaked onto XLD agar (Oxoid code CM469) and incubated at 37°C for 24 hours.

Result: No growth following incubation.

Conclusion: Salmonella spp. absent.

Control: A Salmonella abony culture in tryptone soya broth was streaked onto XLD agar. Red colonies, which developed black centres, grew after incubation at 37°C for 24 hours.

#### 2.4.4 Bioburdens of the tablet excipients

The results of total viable counts showed that potato and wheat starches contained the highest numbers of bacteria and fungi. The numbers of contaminating micro-organisms were within the limits listed in table 1.3, and tests for specified pathogens confirmed



that the excipient powders were of satisfactory microbiological quality.

## 2.5 STERILIZATION OF TABLET EXCIPIENTS

All excipient powders used were sterilized by treatment with ethylene oxide gas (Central Sterile Supplies Department, Guy's Hospital, London) for 12 hours at 45°C and 65% RH, using strips of Bacillus subtilis, var. niger as the biological monitor of sterility. In each case, sterility of the powders was checked prior to use by incubating samples in sterile nutrient broth, and examining them for signs of microbial growth after 18 hours to five days.

## 2.6 BACTERIA

### 2.6.1 The organisms used

The bacteria selected for these studies were Staphylococcus aureus NCTC 10788, and Enterobacter cloacae, a hospital isolate.

The master cultures were 20ml tryptone soya agar slopes of these organisms, stored at 4°C. Table 2.4 lists the cultural and biochemical characteristics of these organisms.

### 2.6.2 Maintaining and using the cultures

From each master culture, a 37°C, 18 hour nutrient broth culture was prepared. The broths were streaked onto nutrient agar plates, incubated at 37°C overnight and examined to check the purity of the cultures. Broth suspensions were then streaked onto 20 x 20ml

Table 2.4 Bacteria selected for study

Organism	Characteristics
<u>Staphylococcus aureus</u>	<p>NCTC strain, 10788.</p> <p>Gram-positive aerobic cocci, non-spore forming, non-motile;</p> <p>catalase - oxidase - O-F test F</p>
<u>Enterobacter cloacae</u>	<p>Glaxo Group Research Ltd. collection, ref. E177 (31.10.85) Source: Papworth Hospital; identity confirmed by API 20E tests.</p> <p>Gram-negative aerobic rods, non-spore forming, motile;</p> <p>catalase + oxidase - O-F test F</p>

tryptone soya agar slopes in screw-capped bottles. Following 18 hours incubation at 37°C, the stock slopes were stored at 4°C, until required. A new stock slope was used each week to prepare working bacterial suspensions as described below, and to prepare additional batches of stock culture as needed.

#### 2.6.3 Preparing working suspensions

A loopful of stock culture was used to inoculate 9ml tryptone soya broth, which was then incubated at 37°C for 18 hours. The purity of this culture was checked by preparing a streak plate on tryptone soya agar. The broth culture (1ml) was used to inoculate the surface of a tryptone soya agar Roux slope in a 300ml flat glass bottle. The slope and streak plates were incubated at 37°C for 18 hours, after which the cells were harvested using 10ml of diluent (peptone buffer). The culture was filtered through sterile gauze to remove lumps of agar and centrifuged at 2500rpm, 0°C for 10 minutes. The supernatant was discarded, and the cells were resuspended in fresh diluent with the aid of an electrical mixer. The washing process was repeated to produce the final working suspension. All of the working suspensions were freshly prepared.

#### 2.6.4 Standardization of the working suspensions

Serial dilutions of the working suspension were prepared, with ultraviolet absorbance values up to 0.5 as measured at 420nm (Cecil Instruments U.V. spectrophotometer, model CE202). The viable count of each dilution was determined as described in



section 2.3.2. Linear plots of U.V. absorbance in peptone buffer versus bacterial viable count were drawn (figures 2.1 and 2.2). These graphs were used to estimate the cell count for subsequent working suspensions, which were then diluted as appropriate with either peptone buffer or water, to give the required number of organisms per ml. Estimated counts of all working suspensions were confirmed by viable counts carried out as before (section 2.3).

Figure 2.1 U.V. absorbance at 420nm for suspensions of  
Ent. cloacae in peptone buffer

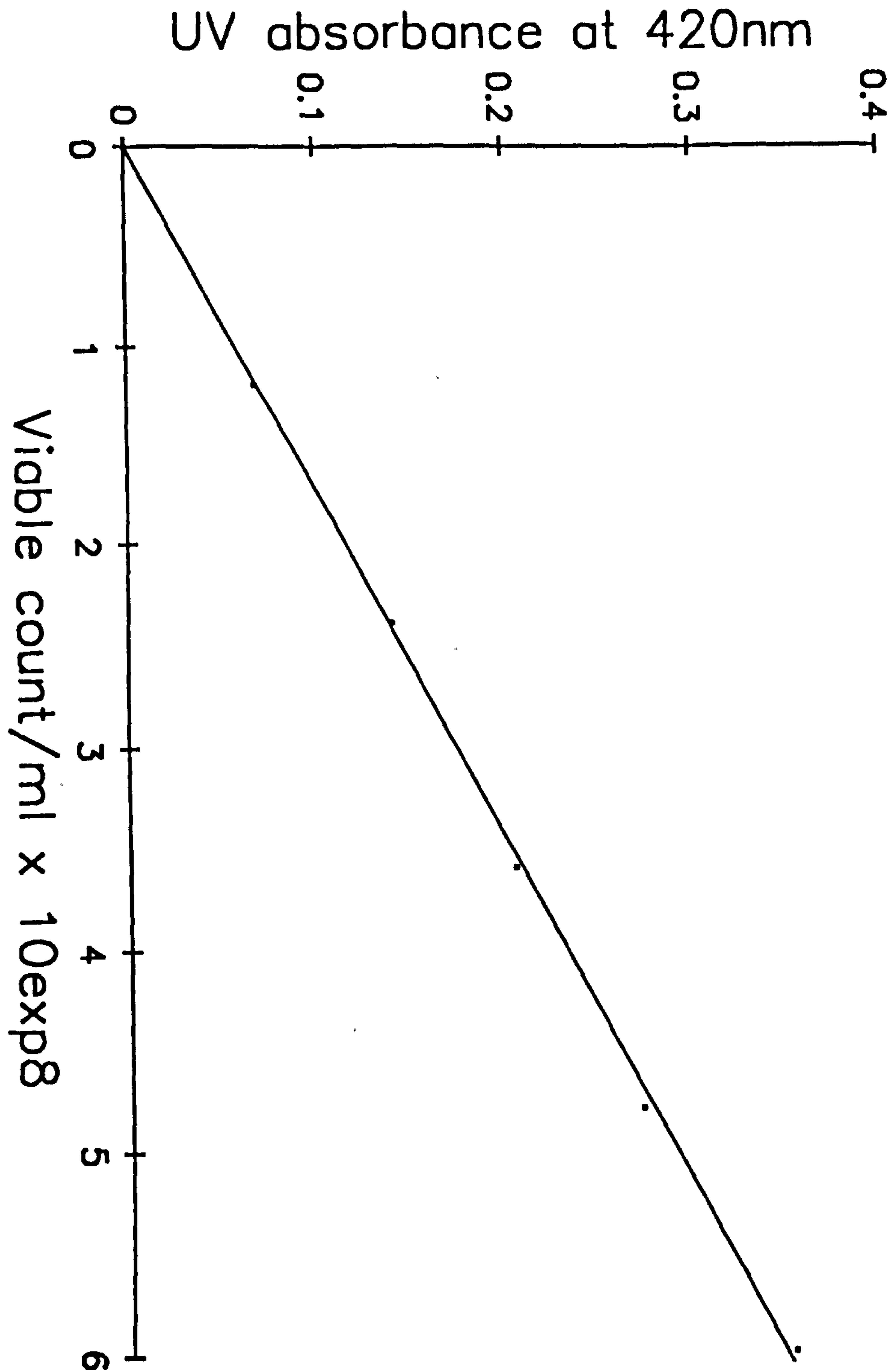
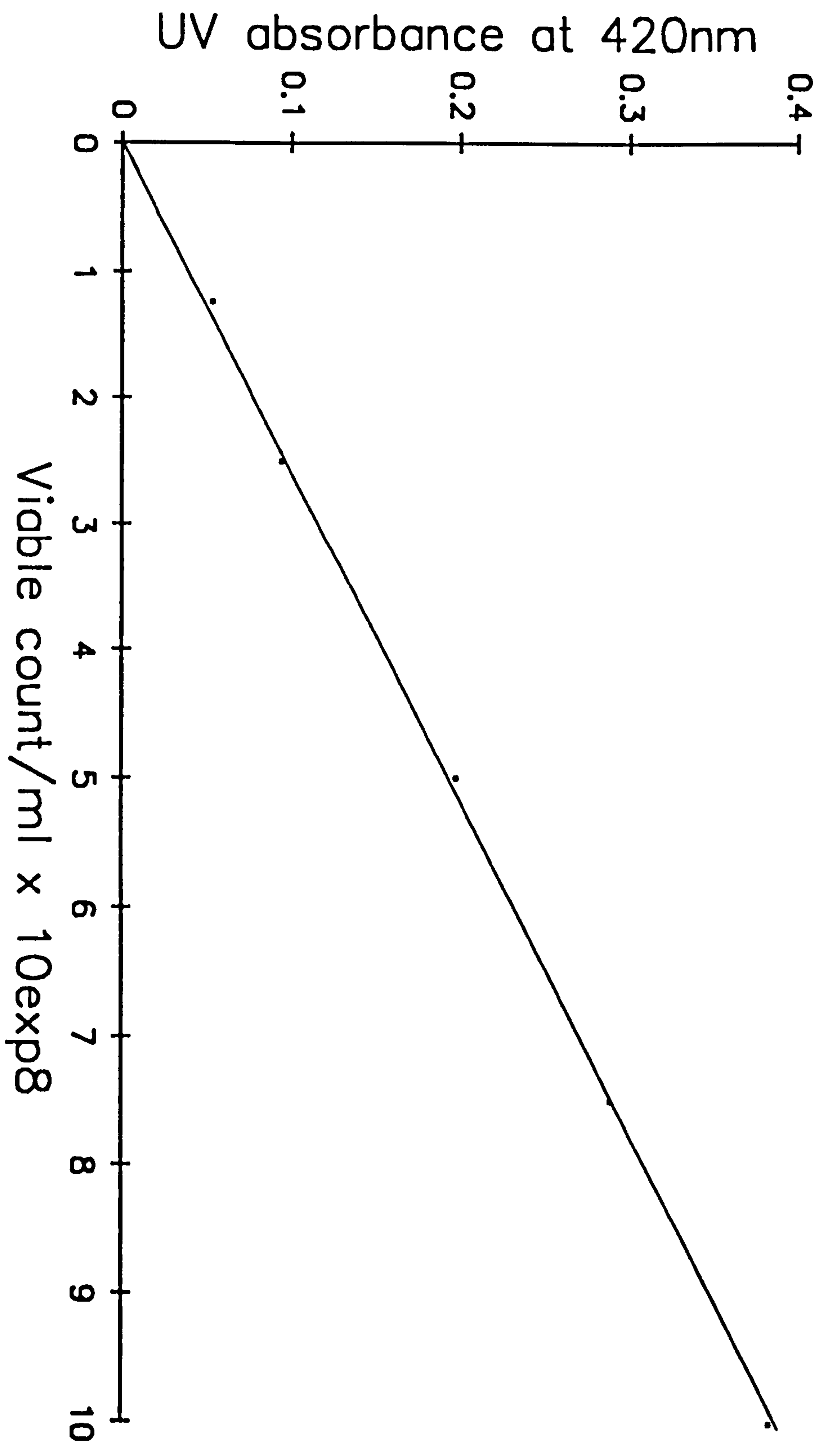


Figure 2.2 U.V. absorbance at 420nm for suspensions of Staph. aureus in peptone buffer





3. PREPARATION OF CONTAMINATED, COMPRESSIBLE EXCIPIENTS

### 3.1 METHODS & RESULTS

#### 3.1.1 Introduction

Contaminated tablets have previously been produced by dry mixing spores with directly-compressible tablet excipients (Fassihi et al, 1977b; Plumpton et al, 1982). However, in the studies that follow, tablet excipients were contaminated with non spore-forming bacteria. In order to inoculate and disperse a population of vegetative cells within tablet excipients, it was necessary to mix the powders with aqueous suspensions of the contaminating organisms. The powder suspensions were then filtered to remove the bulk of the liquid, screened, and finally dried. Since the contaminated excipients were to be used for investigations in which a decline in bacterial survival might be expected, it was necessary to achieve numbers of bacteria within the excipients and tablets far greater than permitted by GMP. In such studies, it was also necessary that the numbers of contaminating organisms should be consistent from batch to batch, to allow for comparisons between different sets of results.

Ishag (1973) attempted to inoculate potato starch with Pseudomonas aeruginosa by mixing the powder with a suspension of bacteria, and filtering off the aqueous phase using a bacteria-proof membrane. Drying of the powder at 37°C resulted in a significant decrease in viable count from  $1.0 \times 10^9$  per gram to  $1.0 \times 10^3$  per gram, after 10 hours. Ishag (1973) considered this method unsatisfactory, and developed a contamination scheme which involved incubating powders and bacteria in the presence of an

aqueous phase. This produced growth of the bacteria within the powder suspension, and high levels of contamination were sustained on powder drying. The same approach has been used for this work.

### 3.1.2 The growth of bacteria in suspensions of tablet excipients

Preliminary studies compared survival and growth of the test organisms in powder/water suspensions or in peptone buffer.

#### Method

Working suspensions of the two test bacteria were prepared as described previously (2.6.3). Sterile distilled water or peptone buffer were used as the diluent for the final working suspension. The working suspension was adjusted to approximately  $10^6$ - $10^8$  colony forming units (cfu) per ml, and was mixed with sterile excipients or diluted further with water or peptone buffer as listed on table 3.1. The test suspensions were incubated at 37°C in sterile plugged 250ml conical flasks, for 48-50 hours. Triplicate 1ml samples were taken at intervals, and the numbers of bacteria determined by the viable counting techniques described in section 2.3.

#### Results

Table 3.2 shows the viable count results, and figures 3.1 and 3.2 show the growth curves obtained by plotting log viable count against incubation time. The results show that the patterns of bacterial survival and growth depended on the organism present, and the nature of the excipient. Ent. cloacae was able to survive or grow in all suspensions with the exception of Avicel PH101



Table 3.1 Suspensions of bacteria and excipients

Excipient or diluent type	Quantity	Suspending agent for bacteria	Quantity
Maize starch	20g	Distilled water	80ml
Wheat starch	20g	Distilled water	80ml
Potato starch	20g	Distilled water	80ml
Avicel PH101	20g	Distilled water	80ml
Avicel CL611	20g	Distilled water	180ml
Lactose	20g	Distilled water	80ml
Distilled water	20ml	Distilled water	80ml
Peptone buffer	20ml	Peptone buffer	80ml

Table 3.2 Growth of bacteria in aqueous suspensions of the test excipients

(i) Enterobacter cloacae

Suspension	Hours at 37°C	Viable count/g	log(viable count/g)
Distilled water	0	$1.78 \times 10^6$	6.25
	4	$1.70 \times 10^6$	6.23
	8	$1.79 \times 10^6$	6.25
	24	$1.74 \times 10^6$	6.24
	50	$2.34 \times 10^6$	6.37
Maize starch	0	$1.78 \times 10^6$	6.25
	4	$2.34 \times 10^7$	7.37
	8	$2.34 \times 10^7$	7.37
	24	$1.24 \times 10^8$	8.09
	50	$3.20 \times 10^8$	8.50
Wheat starch	0	$1.78 \times 10^6$	6.25
	4	$4.39 \times 10^6$	6.64
	8	$4.96 \times 10^6$	6.69
	24	$2.64 \times 10^7$	7.42
	50	$8.79 \times 10^7$	7.94
Potato starch	0	$1.78 \times 10^6$	6.25
	4	$1.21 \times 10^7$	7.08
	8	$1.21 \times 10^7$	7.08
	24	$7.91 \times 10^7$	7.90
	50	$4.02 \times 10^8$	8.60
Avicel PH101	0	$1.78 \times 10^6$	6.25
	4	$1.38 \times 10^6$	6.14
	8	$1.38 \times 10^6$	6.14
	24	$1.21 \times 10^6$	6.08
	50	$1.60 \times 10^5$	5.20
Avicel CL611	0	$1.78 \times 10^6$	6.25
	4	$1.92 \times 10^6$	6.28
	8	$2.22 \times 10^6$	6.34
	24	$6.24 \times 10^6$	6.79
	50	$6.66 \times 10^6$	6.82
Lactose	0	$1.78 \times 10^6$	6.25
	4	$2.18 \times 10^6$	6.34
	8	$2.13 \times 10^6$	6.33
	24	$5.88 \times 10^6$	6.77
	50	$7.69 \times 10^6$	6.89

Table 3.2

(i) Enterobacter cloacae, continued.

Suspension	Hours at 37°C	Viable count/g	log(viable count/g)
Peptone buffer	0	$2.19 \times 10^6$	6.34
	4	$1.94 \times 10^8$	8.29
	8	$2.30 \times 10^8$	8.36
	24	$3.02 \times 10^8$	8.48
	50	$4.54 \times 10^8$	8.66

(ii) Staphylococcus aureus

Distilled water	0	$4.60 \times 10^6$	6.66
	4	$3.13 \times 10^6$	6.49
	8	$5.72 \times 10^5$	5.76
	24	$1.83 \times 10^3$	3.26
	48	< 9	< 0.95
Maize starch	0	$4.60 \times 10^6$	6.66
	4	$4.63 \times 10^6$	6.66
	8	$6.83 \times 10^6$	6.83
	24	$7.80 \times 10^6$	6.89
	48	$5.04 \times 10^5$	5.70
Wheat starch	0	$4.60 \times 10^6$	6.66
	4	$5.30 \times 10^6$	6.72
	8	$4.95 \times 10^6$	6.69
	24	$1.85 \times 10^6$	6.27
	48	$3.30 \times 10^6$	6.52
Potato starch	0	$4.60 \times 10^6$	6.66
	4	$5.65 \times 10^6$	6.75
	8	$1.32 \times 10^7$	7.12
	24	$2.06 \times 10^7$	7.31
	48	$7.16 \times 10^6$	6.85
Avicel PH101	0	$4.60 \times 10^6$	6.66
	4	$1.69 \times 10^6$	6.23
	8	$8.64 \times 10^5$	5.94
	24	44	< 1.64
	48	< 8	< 0.90
Avicel CL611	0	$4.60 \times 10^6$	6.66
	4	$1.96 \times 10^6$	6.29
	8	$1.20 \times 10^6$	6.08
	24	$5.13 \times 10^5$	5.71
	48	$9.17 \times 10^5$	5.96



Table 3.2

(ii) Staphylococcus aureus, continued.

Suspension	Hours at 37°C	Viable count/g	log(viable count/g)
Lactose	0	$4.60 \times 10^6$	6.66
	4	$7.40 \times 10^5$	5.87
	8	$2.65 \times 10^4$	4.72
	24	$1.03 \times 10^3$	3.01
	48	< 8	< 0.90
Peptone buffer	0	$2.65 \times 10^6$	6.42
	4	$6.15 \times 10^6$	6.79
	8	$2.28 \times 10^7$	7.35
	24	$7.57 \times 10^7$	7.88
	48	$7.60 \times 10^7$	7.88

Figure 3.1 Growth of Ent. cloacae in peptone buffer and in aqueous suspensions of the test excipients

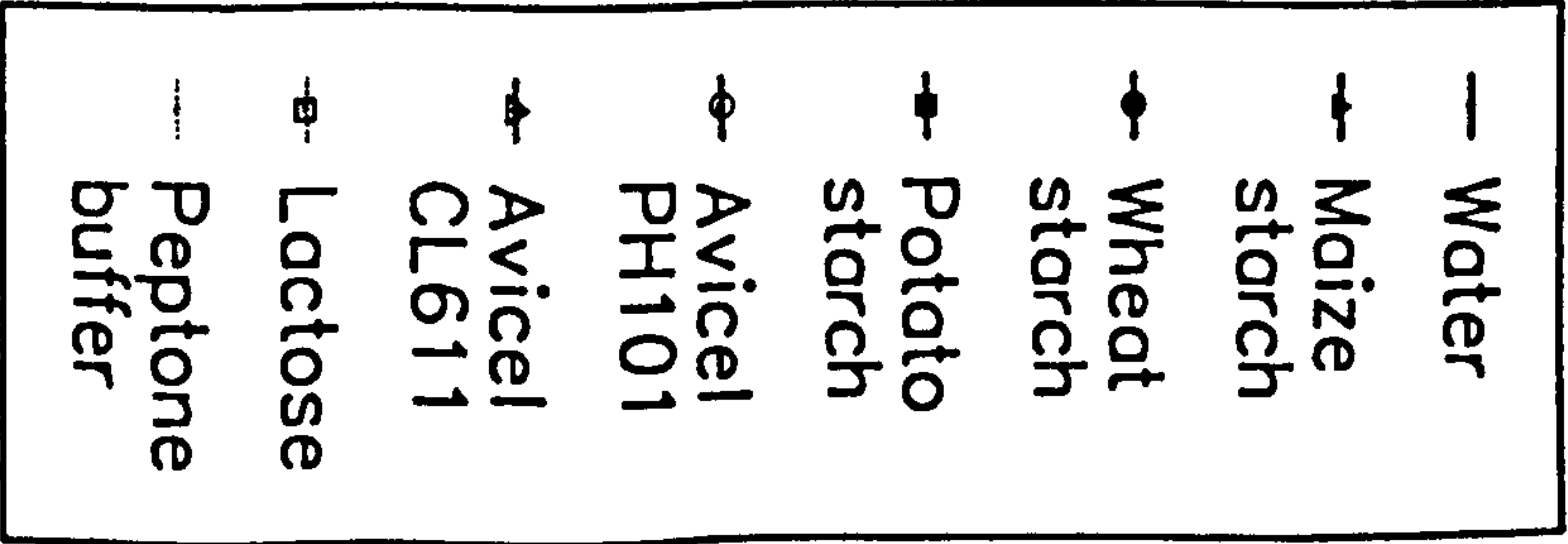
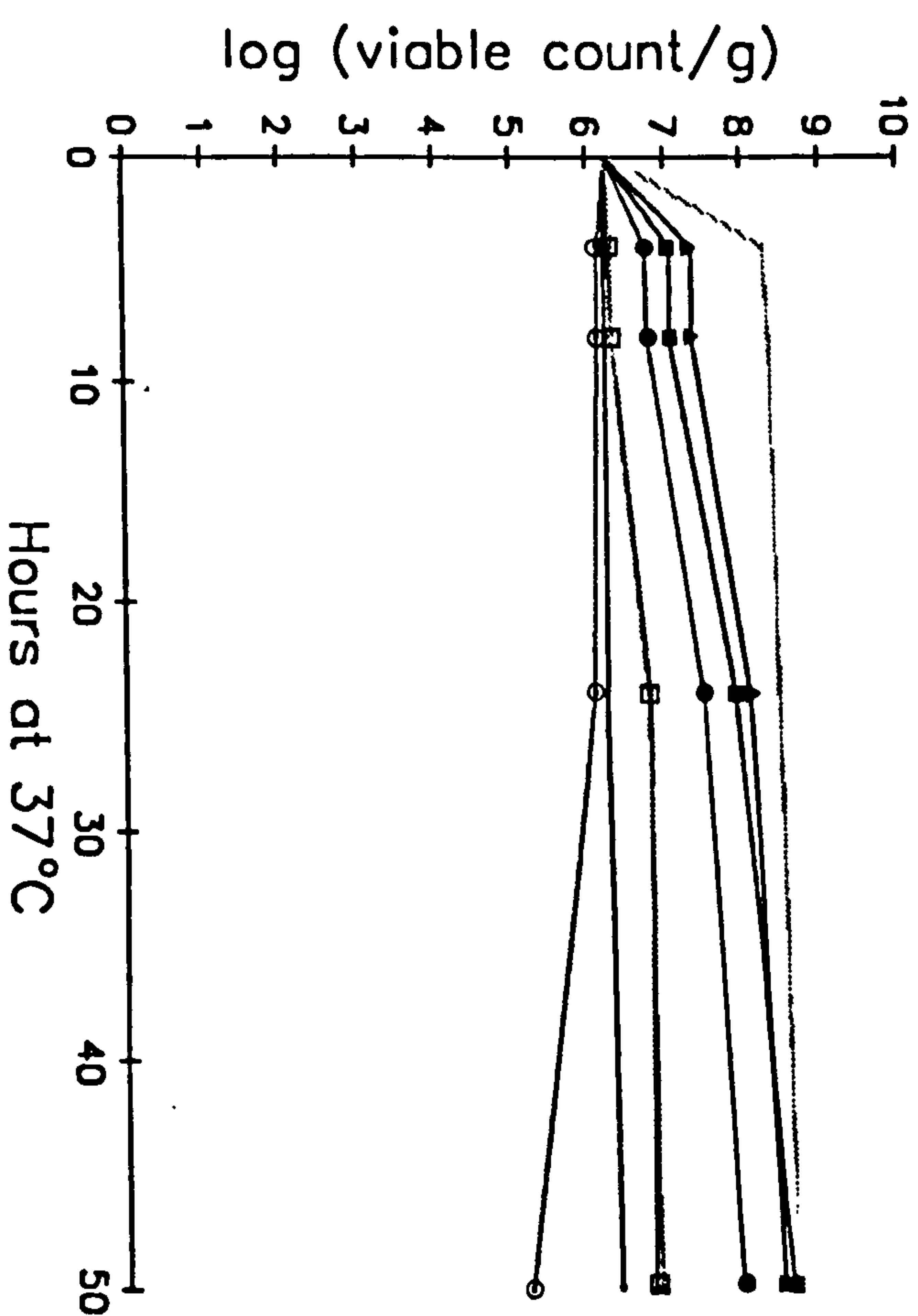
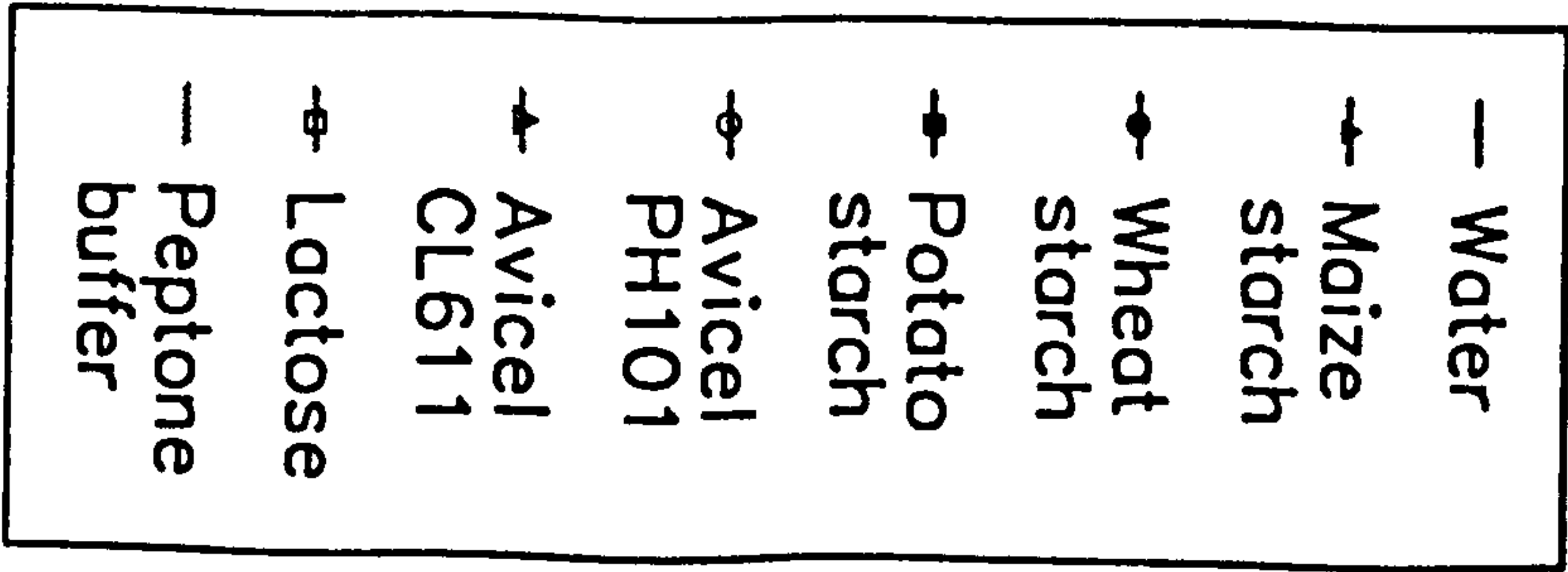
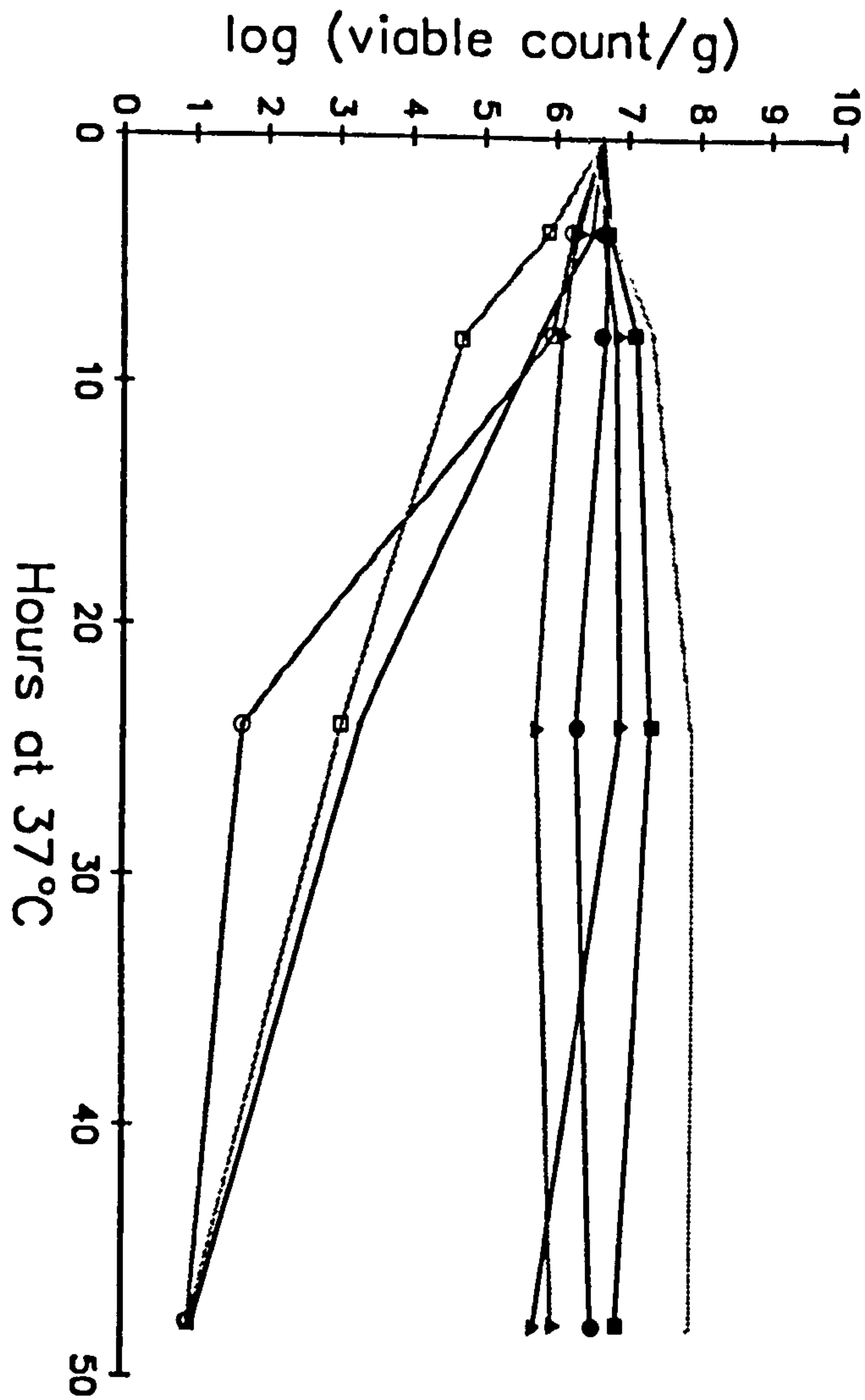


Figure 3.2 Growth of Staph. aureus in peptone buffer and in aqueous suspensions of the test excipients





(microcrystalline cellulose), and grew particularly well in starch suspensions. Staph. aureus was unable to grow in distilled water, or in suspensions of Avicel PH101 or lactose. Both organisms grew well in suspensions of peptone buffer.

As stated previously, in contaminating the test excipients the aim was to achieve comparable, high levels of microbial contamination, able to persist beyond the tableting stage. Since the incubation of excipients with aqueous suspensions of the test bacteria gave variable results peptone buffer suspensions were used for all further studies to initiate growth of contamination.

### 3.1.3 Preparation of contaminated excipients

The bulk of the aqueous phase had to be removed to produce directly-compressible granules from the contaminated powder suspensions. This was followed by careful drying to preserve the viability of the contaminating bacteria. Preliminary experiments indicated that organisms in contaminated granules survived better when dried slowly at room temperature than at 37°C, as used by Ishag (1973).

#### Method

Working suspensions of the bacteria in peptone buffer (200ml) were mixed with each of the sterile excipients (60g), and incubated at 37°C for 48 hours. The suspensions were then filtered to remove the bulk of the liquid, using a 500ml vacuum filter flask (Sartorius) fitted with a 0.45 $\mu$ m filter (Millipore). The damp excipient powders were screened to 10 mesh size, spread onto pyrex trays under a sterile, laminar flow of air, and allowed to dry at

room temperature. Two weighed samples, of about 1g, were taken hourly, and duplicate viable counts were carried out by the methods described in section 2.3. The loss of water from the damp powders was followed by measuring the relative humidity of hourly samples at 25°C using a Rotronic BT Hygroskop (WA-14TH).

### Results

Table 3.3 shows the viable counts of the powder suspensions before incubation, the filtered wet powders after incubation, and the powders after drying for 8 hours. Drying was most rapid for wet lactose, followed by Avicel PH101, potato starch, and then the other starches. Survival during drying depended on the contaminating organism, and the rate at which water was lost from each powder. Typical surviving/drying patterns are shown in figure 3.3. Ent. cloacae was particularly sensitive to drying, and a drop in viable count reflected the reduction in relative humidity. Staph. aureus survived well as the powders dried. Some of the materials used were resistant to drying by this method. They included Avicel grades RC581, RC591 and CL611, which are microcrystalline cellulose co-processed with sodium carboxymethylcellulose (sodium CMC). The presence of sodium CMC makes it more difficult to remove water from the microcrystalline cellulose. Since more rigorous drying methods would reduce the yield of bacteria in the contaminated excipients, the powders with sodium CMC were excluded from studies using contaminated "dry" excipients, but were involved in later investigations of the interactions between excipients and water vapour.

Table 3.3 Viable counts of bacteria at various stages of the contamination process

(i) Ent. cloacae

Excipient	Working suspension + excipient cfu/ml before incubation	Filtered, wet excipients cfu/g after incubation	Excipients cfu/g after drying
Avicel PH101	$3.4 \times 10^8$	$7.1 \times 10^8$	$3.5 \times 10^8$
Avicel CL611	$2.0 \times 10^8$	$2.2 \times 10^8$	$4.6 \times 10^8$
Avicel RC581	$1.5 \times 10^8$	$4.2 \times 10^8$	$7.5 \times 10^8$
Avicel RC591	$3.3 \times 10^8$	$1.3 \times 10^8$	$1.4 \times 10^8$
Maize starch	$3.7 \times 10^8$	$2.4 \times 10^9$	$9.3 \times 10^7$
Wheat starch	$2.7 \times 10^8$	$1.0 \times 10^{10}$	$3.7 \times 10^9$
Potato starch	$3.8 \times 10^8$	$7.1 \times 10^8$	$5.4 \times 10^7$
Lactose	$1.4 \times 10^8$	$8.4 \times 10^9$	$3.1 \times 10^7$

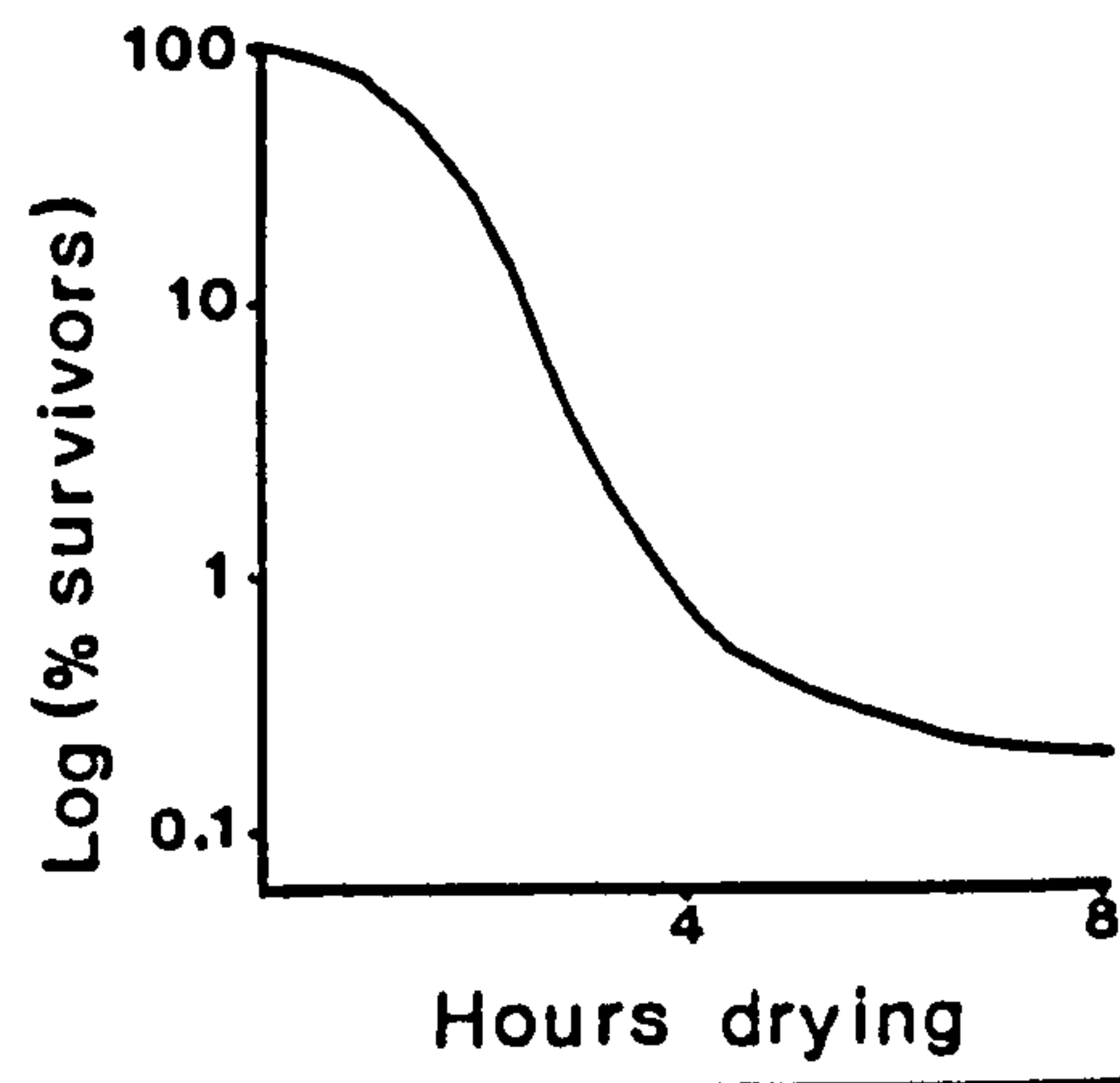
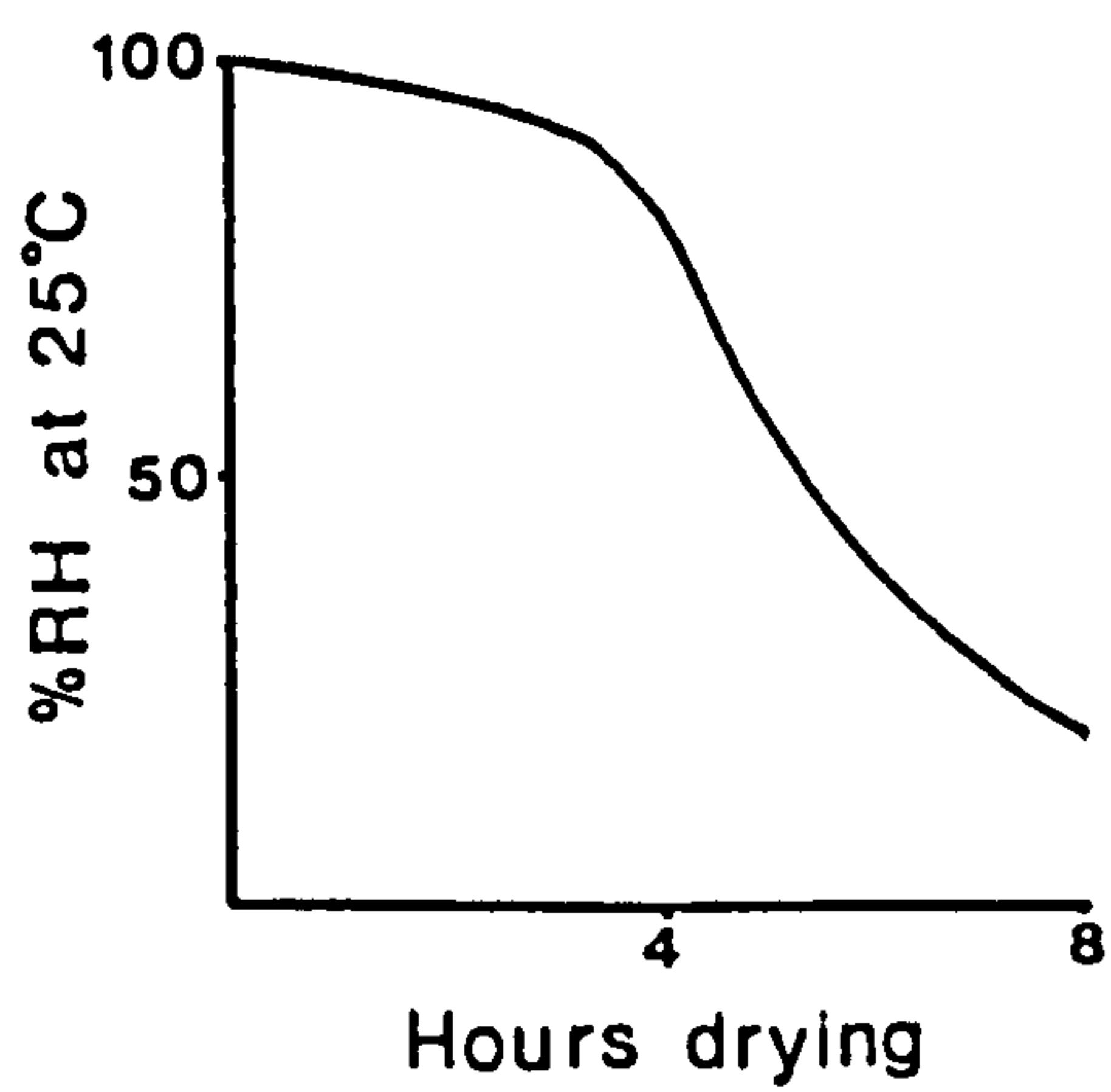
(ii) Staph. aureus

Excipient	Working suspension + excipient cfu/ml before incubation	Filtered, wet excipients cfu/g after incubation	Excipients cfu/g after drying
Avicel PH101	$4.5 \times 10^8$	$1.8 \times 10^9$	$9.7 \times 10^8$
Avicel CL611	$7.4 \times 10^7$	$8.3 \times 10^7$	$1.1 \times 10^8$
Avicel RC581	$9.1 \times 10^7$	$7.7 \times 10^8$	$8.5 \times 10^8$
Avicel RC591	$5.5 \times 10^7$	$1.1 \times 10^8$	$1.6 \times 10^8$
Maize starch	$6.2 \times 10^8$	$2.1 \times 10^9$	$2.3 \times 10^9$
Wheat starch	$1.1 \times 10^8$	$2.9 \times 10^9$	$2.7 \times 10^9$
Potato starch	$7.2 \times 10^7$	$2.8 \times 10^8$	$2.6 \times 10^8$
Lactose	$8.3 \times 10^7$	$7.6 \times 10^8$	$5.6 \times 10^8$

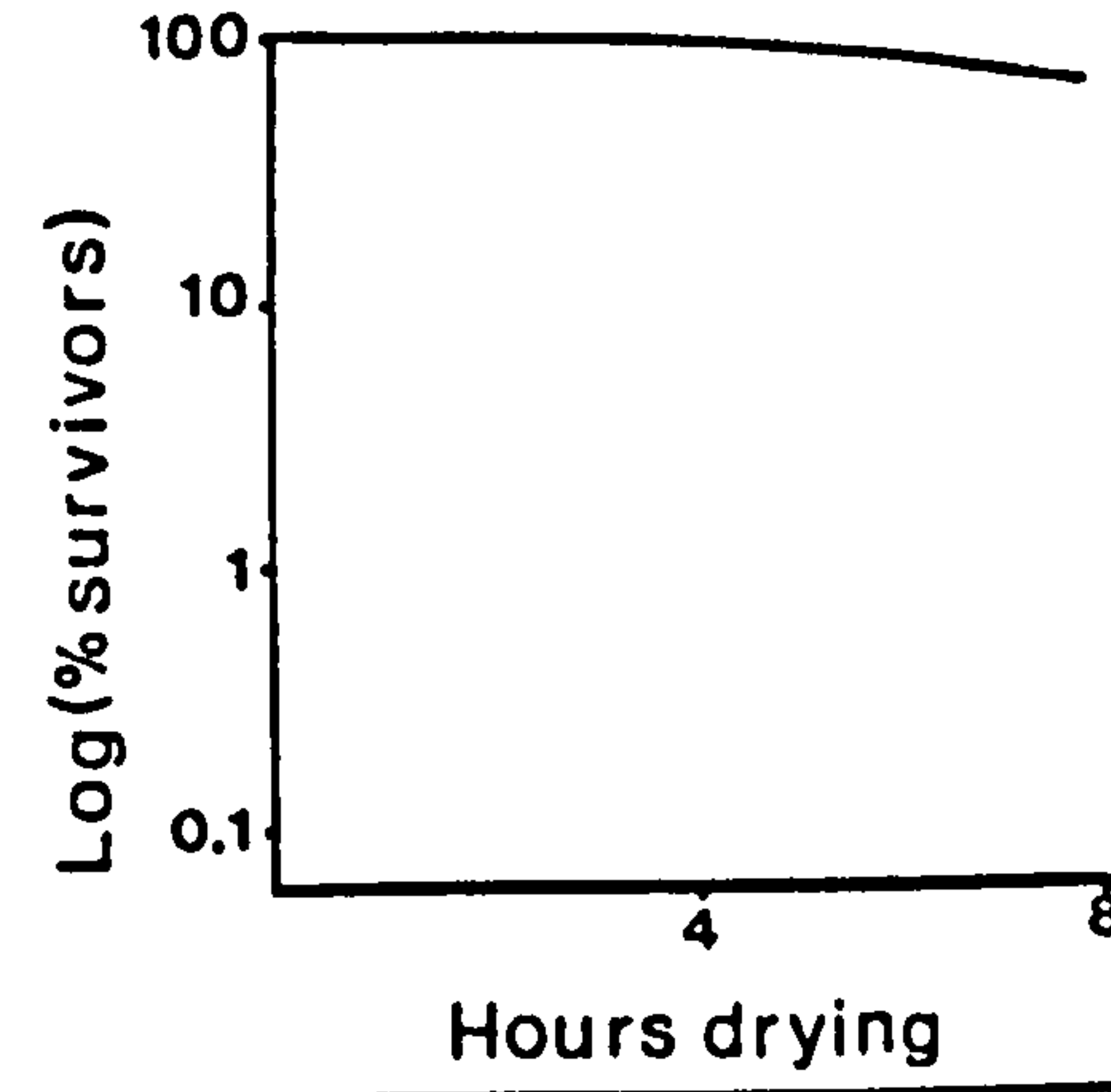
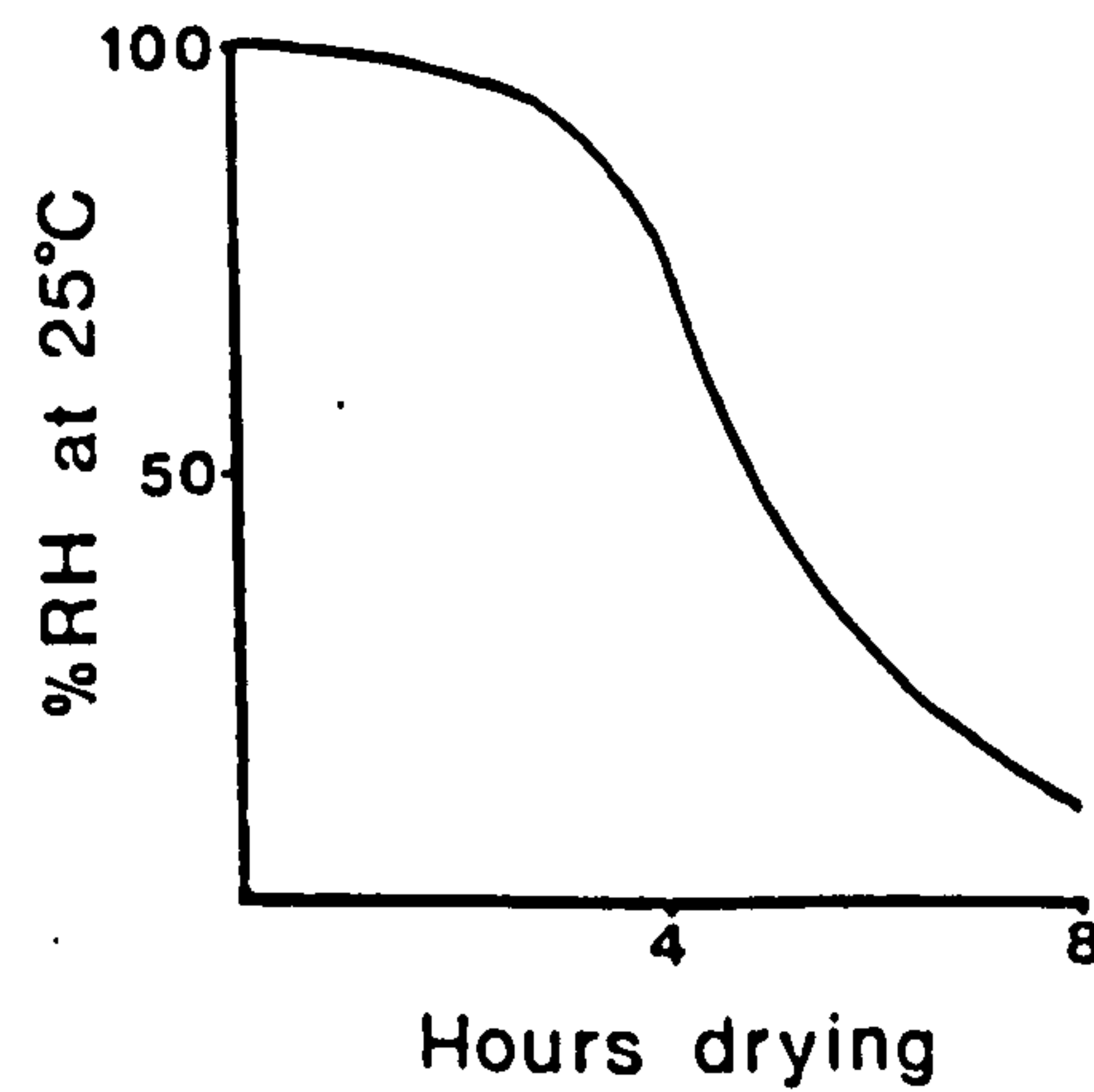


Figure 3.3 Three patterns for bacterial survival and relative humidity for contaminated excipients dried under a constant flow of air at room temperature.

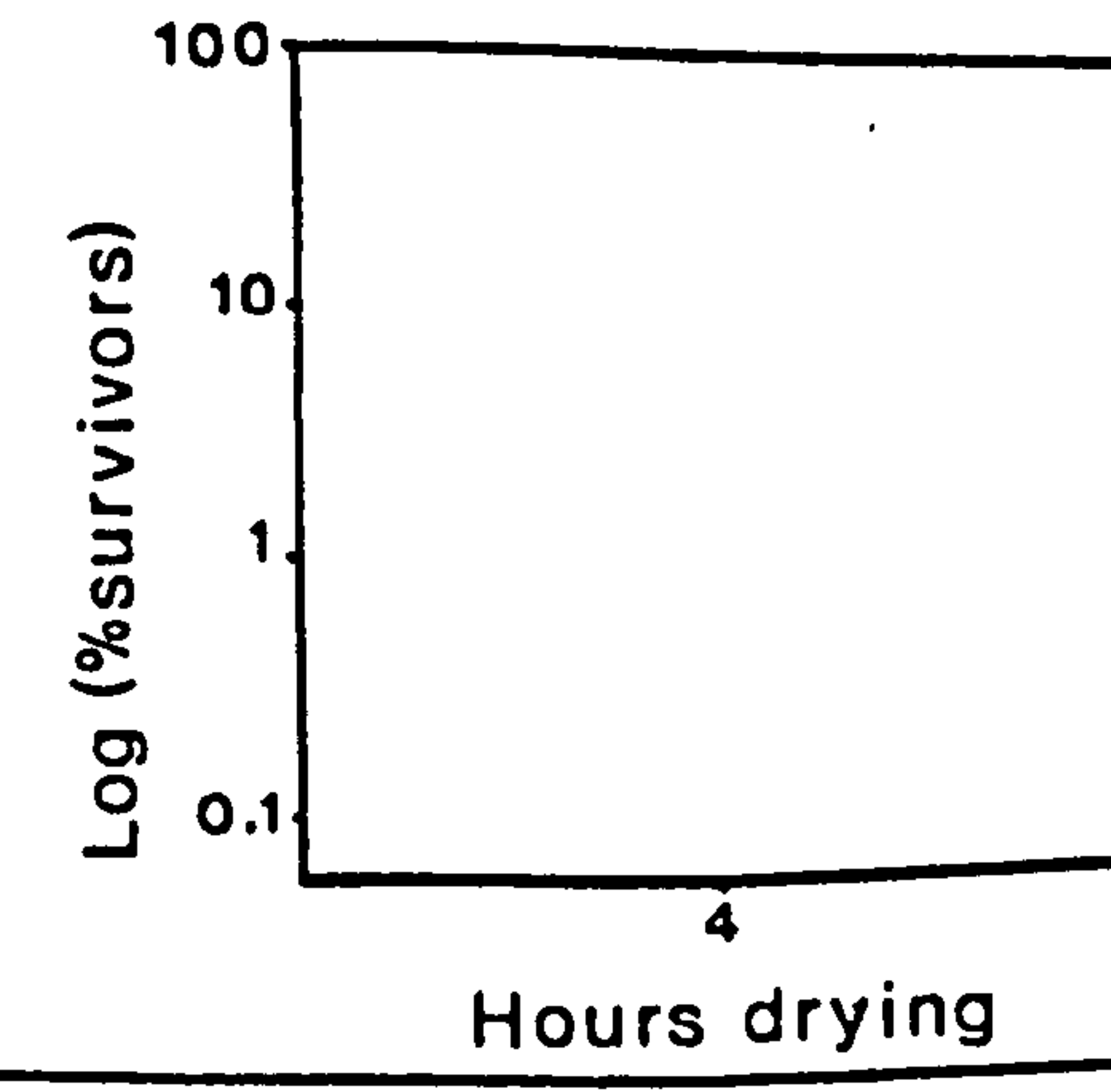
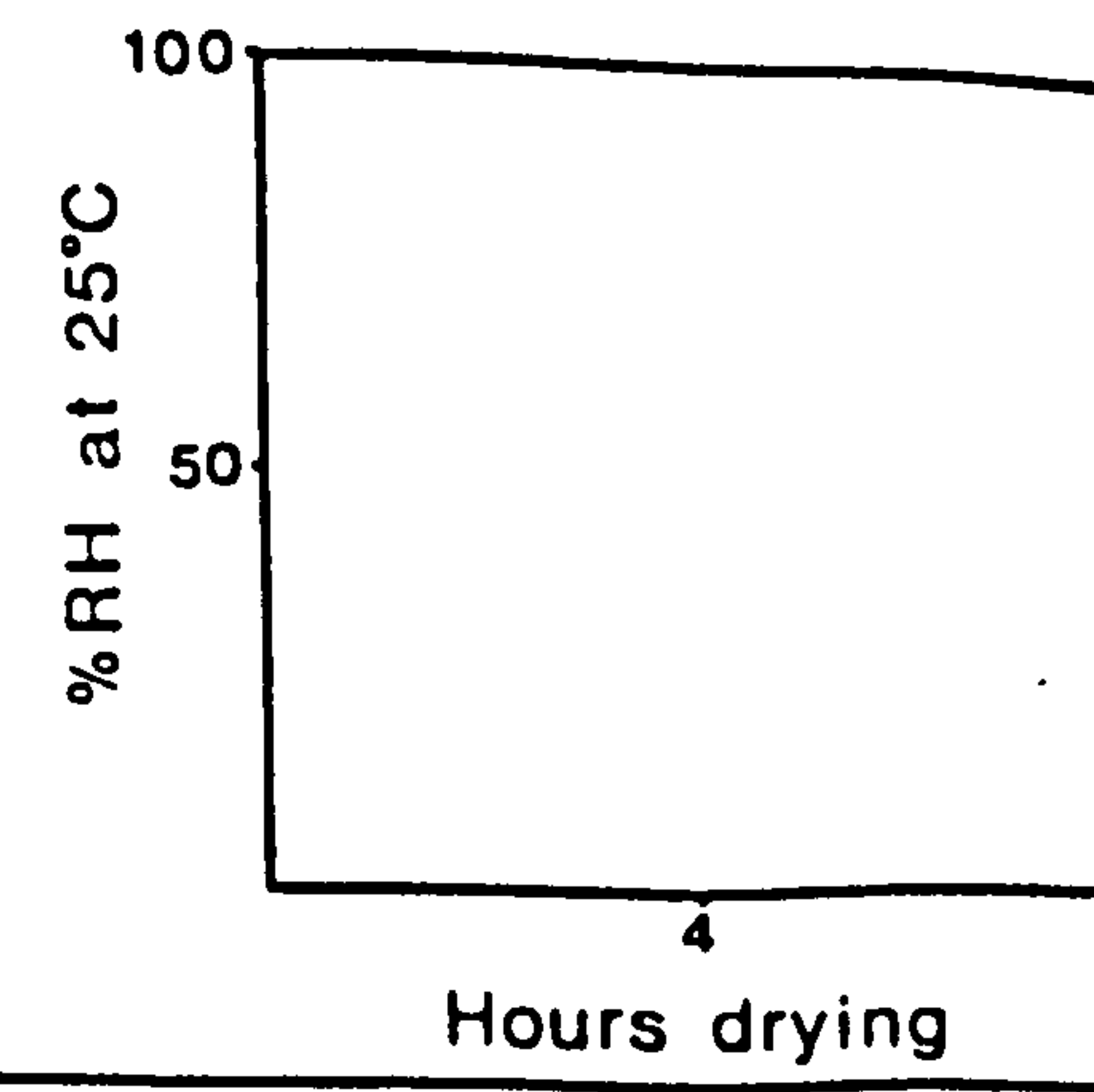
(i) Drop in viable count mirrors the drop in relative humidity. Typical of Ent. cloacae in Avicel PH101, the starches and lactose.



(ii) Organisms show resistance to drying. Typical of Staph. aureus in Avicel PH101, the starches and lactose.



(iii) Excipients resistant to drying. Typical of Avicel grades with sodium carboxymethylcellulose.



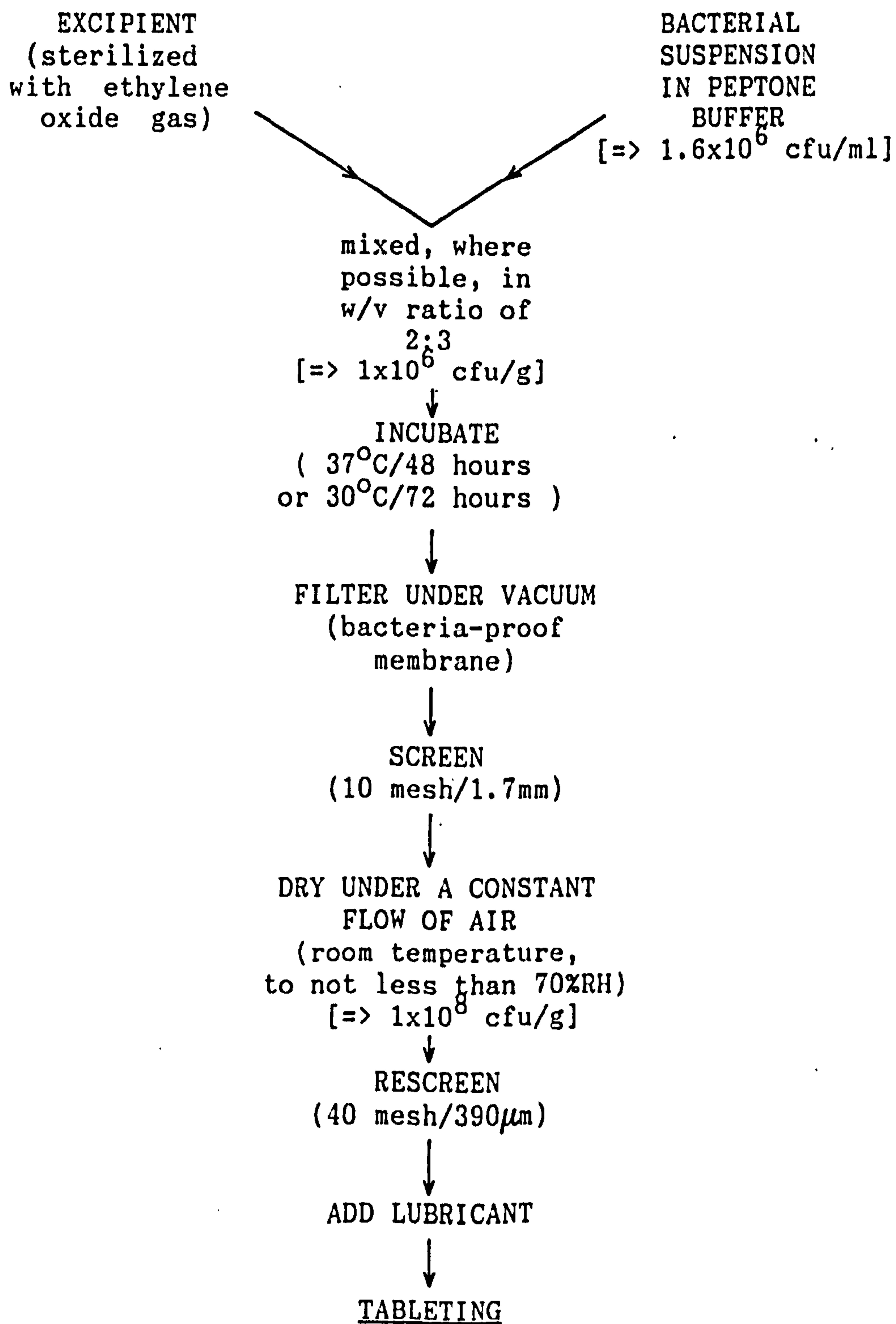
#### 3.1.4 Scheme for producing contaminated, directly-compressible tablet excipients

From a consideration of the results of 3.1.3, the following method was adopted for producing contaminated directly-compressible tablet excipients. The contamination scheme is shown in figure 3.4.

##### Method

Working suspensions in peptone buffer were adjusted to contain approximately  $1.6 \times 10^6$  cfu per ml (unless specified otherwise). The working suspension was in each case mixed with sterile excipient powder, in the ratio of 3 parts suspension to 2 parts powder. Typical quantities were 180ml suspension with 120g powder. This ratio of 3:2 gave an initial viable count for the powder suspension of about  $1 \times 10^6$  cfu per ml. Incubation (for 48 hours at  $37^\circ\text{C}$  or 72 hours at  $30^\circ\text{C}$ ) resulted in powder suspensions with high viable counts between  $10^7$  and  $10^9$  cfu per ml, some of which would be lost on drying. The powder suspensions were filtered and prepared for drying as described above (section 3.1.2). The excipients were dried to 70% RH, which gave "dried" excipients of not less than about  $10^5$  cfu per gram. The excipients were re-screened to 40 mesh size (i.e. granules no bigger than  $390\mu\text{m}$  diameter), and sterile magnesium stearate (1% w/w) was added by increasing quantities, to act as lubricant. The contaminated powders were transferred to sterile screw-capped jars and used for tableting studies.

Figure 3.4 Scheme for producing contaminated, directly-compressible tablet excipients





### 3.1.5 Scanning electron microscopy

Samples of the excipients were gold-coated and examined by scanning electron microscopy as before (section 2.4.1). The printed micrographs (plate 3) show Ent. cloacae and Staph. aureus attached to maize starch and Avicel PH101.

## 3.2 DISCUSSION OF RESULTS

The literature (as reviewed in section 1.5) reports a number of studies of micro-organisms processed in solid oral dosage forms, but very few studies involving vegetative bacteria. It may be argued that vegetative bacteria are not important in solid oral dosage forms because of kill during granule drying. However, current and future trends to use directly-compressible formulations, or alternative methods of tablet processing which avoid high temperature drying, demand that we should be concerned about possible problems associated with the survival of vegetative as well as spore-forming micro-organisms.

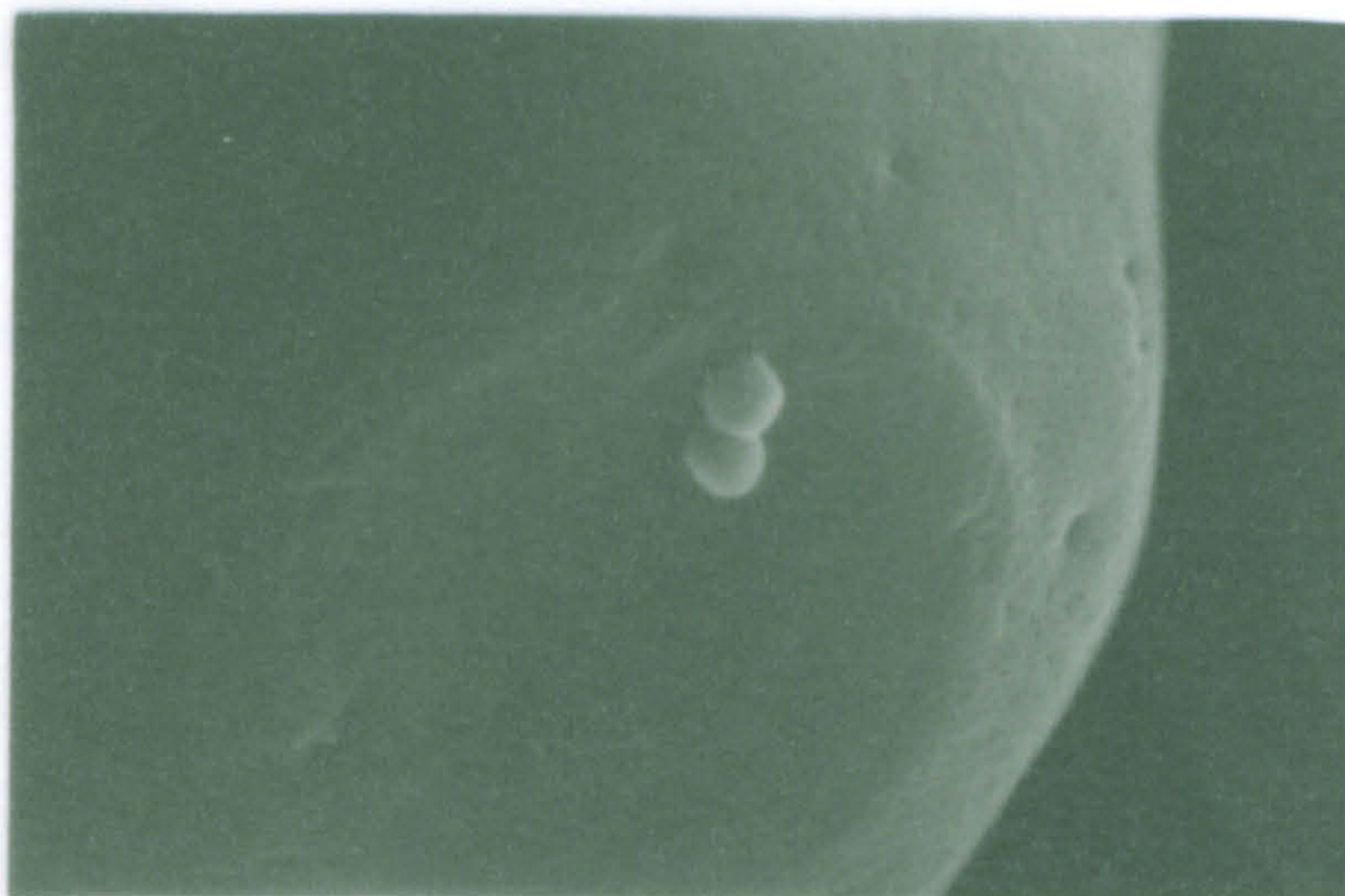
To study bacterial survival during tablet processing, the first requirement was to produce dry, directly-compressible excipients, contaminated with viable vegetative bacteria. It was also necessary that the levels of contaminating micro-organisms in the dry excipients should be high enough to facilitate subsequent investigations of the effects of tablet processing, and survival on storage.

A contamination method similar to that adopted by Ishag (1973) was adopted. Preliminary studies were carried out to investigate

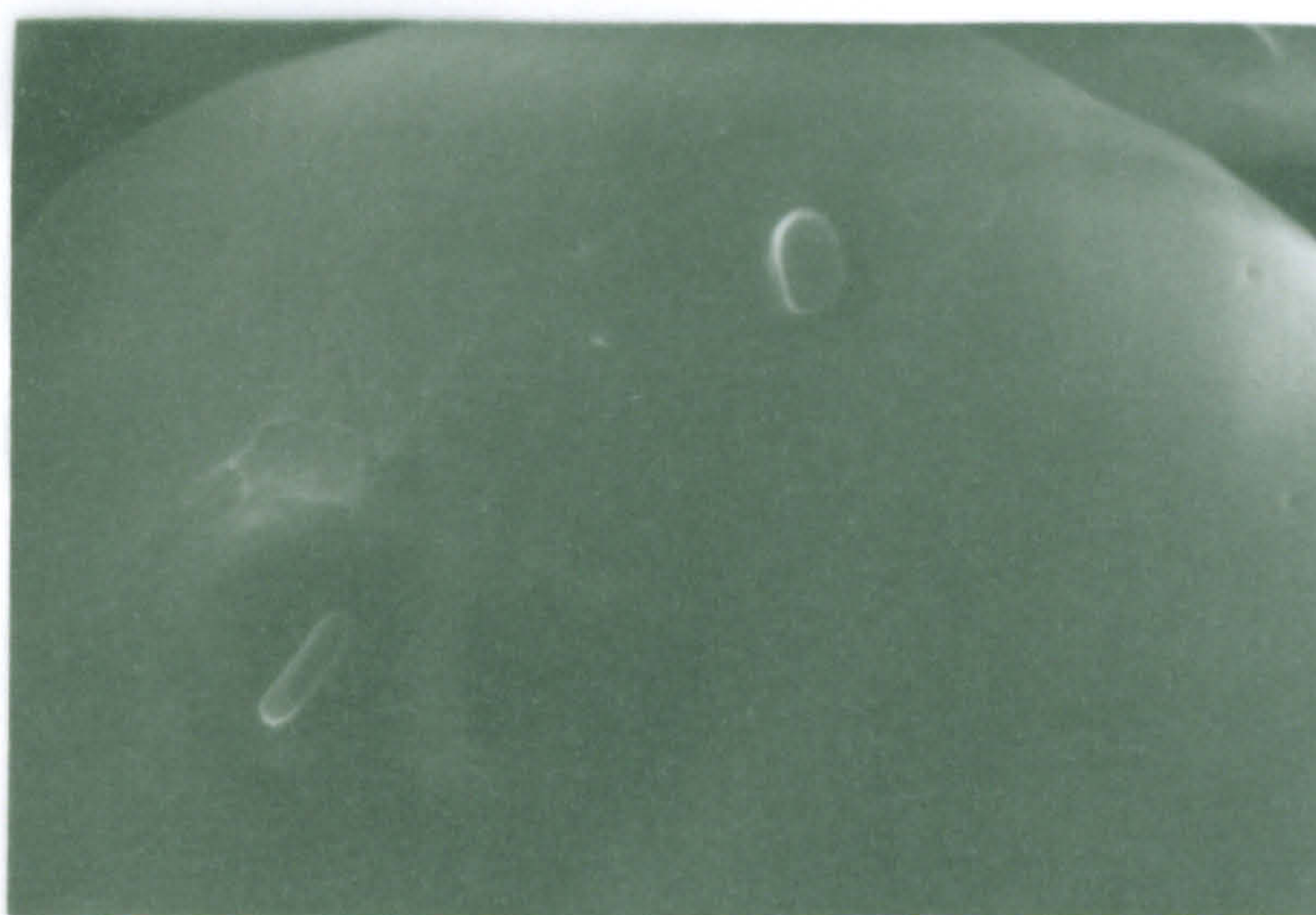


Plate 3. Scanning electron micrographs of tablet excipients  
showing contaminating micro-organisms

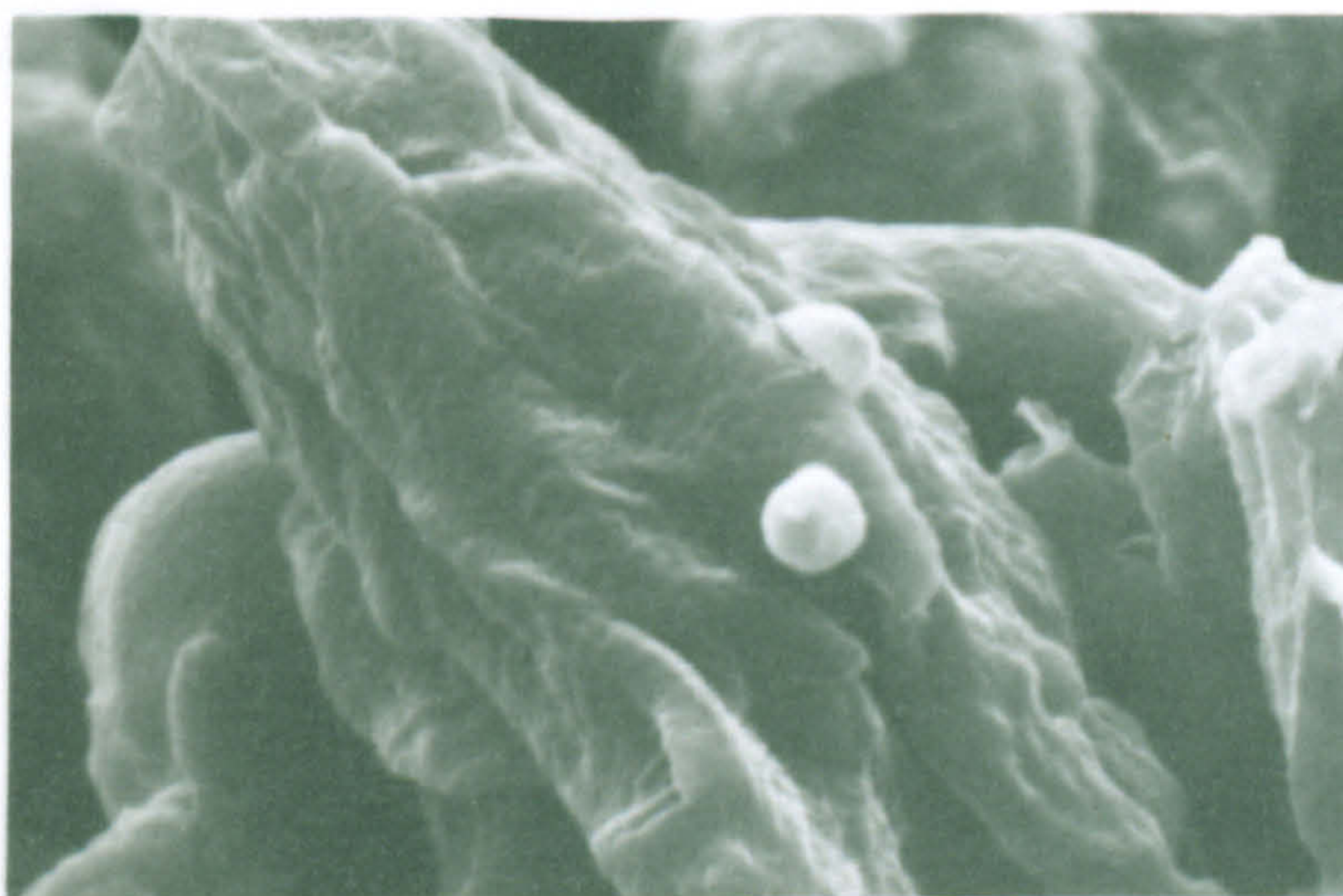
(a) Maize starch, with Staph. aureus (x6900)



(b) Maize starch, with Ent. cloacae (x6900)



(c) Avicel PH101, with Staph. aureus (x6900)





bacterial growth supported by the various excipients, by incubating the test bacteria in suspensions of the excipients in distilled water. Bacterial growth curves for Ent. cloacae and Staph. aureus at 37°C are shown in figures 3.1 and 3.2.

Ent. cloacae, which is not very nutritionally-exacting, was able to survive well in each of the test systems and also in distilled water. This is not surprising, since it has previously been observed that Ent. cloacae is able to grow to  $10^6 - 10^7$  organisms per ml in distilled water or 0.001% peptone water (Leak, 1983). In our investigations, Staph. aureus survived in some but not all of the powder-water systems. Ishag (1973) similarly found that Staph. aureus showed less growth than Ps. aeruginosa or E. coli in aqueous excipient suspensions.

Ent. cloacae is a lactose-fermenting organism, and it was surprising that the growth of this organism in the lactose-water system was relatively poor. Staph. aureus however, does not have the ability to utilise lactose, which may explain the loss of viability in the lactose system.

The starches supported growth of Ent. cloacae and, to a lesser extent, Staph. aureus. Cellulosic materials like starch are made up of monomer building blocks of anhydroglucose. Bacteria can break down the cellulose into shorter anhydroglucose chains, until glucose is eventually liberated. The fact that celluloses, particularly those of natural origin, are good sources of nutrients for bacteria, may explain the frequency of literature reports concerned with contamination in natural gums and starches



(Kallings et al, 1966b; Knusel and Hess, 1968; Pedersen and Ulrich, 1968; Schiller et al, 1968; Wanandi and Speiser, 1970).

It is interesting to compare the behaviour of the test bacteria in aqueous suspensions of Avicel PH101 and Avicel CL611. The suspension of Avicel PH101, which is insoluble microcrystalline cellulose, appears to be the least favourable environment for the survival or growth of both organisms. However, for Avicel CL611, which is microcrystalline cellulose co-processed with water-soluble sodium CMC, much better growth was observed for both Ent. cloacae and Staph. aureus. It is well known that solutions of sodium CMC support microbial growth, and the use of bacteriostats in aqueous cellulose solutions has been advocated (Freeman et al, 1948). More recent studies have shown that sodium CMC is a particularly good substrate for microbial growth when the degree of substitution (DS) is less than 1.2 (Banker et al, 1982). The sodium CMC used to produce Avicel CL611 has a DS of 0.7, and would thus be expected to provide a ready source of nutrition for microbial growth.

The peptone buffer used in these investigations was based on an isotonic phosphate buffer of neutral pH. Observations by Buhlmann (1968) indicated that the addition of 0.1% peptone to a phosphate buffer of the same composition improved the germination of spores and survival of vegetative and spore-forming bacteria, especially in the case of damaged cells. This had the effect of increasing the yield of microbial contaminants isolated from non-sterile pharmaceuticals under test. Results shown in figures 3.1 and 3.2

indicate that the growth of both Ent. cloacae and Staph. aureus in the peptone buffer solution, compared favourably with growth or survival in any of the excipient/water systems. Using peptone buffer suspensions of excipient powders, it was possible to achieve consistently high levels of contamination (in the order of  $10^8$  cfu per ml, and up to  $10^{10}$  cfu per ml in some cases) in powder suspensions following incubation.

In order to produce directly compressible excipients for tableting, the aqueous peptone buffer phase was removed by filtration, and the damp powders screened and dried. Ishag (1973) reported that contaminated powder suspensions could be dried at  $37^{\circ}\text{C}$  without killing all of the contaminating bacteria, but our preliminary studies indicated that bacterial survival was better following slow drying in an air flow at room temperature than more rapid drying at  $37^{\circ}\text{C}$ . This indicates that unlike moulds, bacteria are less tolerant of reduced  $A_w$  at temperatures close to optimum growth, and that viability is better preserved when the drying rate is slow. This agrees with the findings of Bullock and Lightbrown (1947), who attributed the lethal effect of drying to the rapid removal of water from the cells.

An investigation of the survival of bacteria in contaminated powders dried at room temperature (figure 3.3 and table 3.3) confirmed that bacterial survival on granule drying was dependent upon both the rate of water removal from the granules, and the drought-tolerance of the organisms. For Ent. cloacae, the loss in viability during granule drying was greatest in lactose and Avicel



PH101, which were the excipients from which water loss was most rapid. Whilst death of Ent. cloacae correlated with the water loss from the excipients, Staph. aureus was relatively tolerant of water loss from the granules. This illustrates the difference in water requirements of the two organisms. According to Troller and Christian (1978) the Gram negative rod Ent. cloacae, has a minimum  $A_w$  requirement for growth, of 0.96-0.94 $A_w$ , whereas Staph. aureus strains have much wider  $A_w$  tolerance, and have been shown to grow between 0.86 and 0.83 $A_w$  (Troller and Christian, 1978; Hill, 1973 unpublished, through Tatini, 1973). Furthermore, the staphylococci have some ability to compensate for the effects of reductions in  $A_w$ , by the accumulation of low molecular weight solutes from the menstruum, which allow the cytoplasm to adjust to a lower  $A_w$  (Christian and Waltho, 1962). Slower drying measures, as used in these studies, may give Staph. aureus more time to adjust to the lowered  $A_w$ .

The nature of the excipients markedly affects the behaviour of bacteria during drying. This infers an intimate relationship between powders, microbial cells and available water. Examination of the dried, contaminated granules by scanning electron microscopy showed bacterial cells individually or in pairs, adhering at the solid surface, and it is assumed that the cells had become attached to the particles during the incubation period.

The mechanisms of microbial adhesion to solids have been studied. Suspensions of cells behave as "living colloids" (Marshall and Bitton, 1980), adsorbing to larger particles initially by weak



attraction such as Van der Waals or hydrophobic forces. Following this initial reversible sorption of cells onto larger particles in suspension, the micro-organisms attach to the solid surface in a non-specific and irreversible manner, by secreting polymeric adhesives. If favourable conditions for growth persist, an adherent microcolony develops at the solid-liquid interface, cemented on by extracellular polymer. According to Costerton et al (1981), adherent microcolonies "are the predominant form of bacterial growth in nature". Starches and celluloses are digested by adherent microcolonies (Costerton et al, 1981).

The ability to grow on surfaces is advantageous to bacterial cells for several reasons. Nutrient materials, in the form of macromolecules, colloids and ions, tend to concentrate at the solid-liquid interface. Furthermore, attachment at the solid surface allows for reproducible growth within the controlled environment of the microcolony (Brown and Williams, 1985). In a dry environment, the macromolecular nutrients surrounding the bacteria will tend to have a protective effect against dehydration (McEldowney and Fletcher, 1988).

Scanning electron micrographs (plate 3) showed that the contaminating bacteria had become attached to the particle surfaces, although extensive microcolonies were not observed. It was not possible to determine any detail of the structures mediating bacterial attachment, but Costerton (1980) also noted that scanning electron microscopy has limited resolution in this respect. Bacteria attached to the powders in this way are likely

to be more resistant to harmful external effects such as drought. It is suggested that the method of powder contamination adopted for these investigations produced micro-organisms intimately associated with the substrate powders, and in a condition more closely related to the situation for naturally-contaminated, dry materials than those used in previous studies.

4. THE PREPARATION OF TABLETS FROM CONTAMINATED EXCIPIENTS



## 4.1 METHODS AND RESULTS

### 4.1.1 Introduction

Compaction mechanisms involved in tableting, and the effect of tableting on contaminating micro-organisms were reviewed in section 1.5.3. There have been no reports of the effect of tablet compression on vegetative bacteria grown within the excipient powders. In this part of the investigation, bacterial inactivation was studied, during the compression of contaminated excipients into tablets. The results were also used to identify levels of compression force which gave suitably non-friable tablets and allowed sufficient bacteria to survive for subsequent studies of the contaminated tablets.

### 4.1.2 The instrumented tablet machine

Tablets with an approximate weight of 330mg (+/- 20mg) were prepared using a Manesty F3 single punch machine (Manesty Machines Ltd.), with 10.5mm flat-faced punches. The machine surfaces and dies were disinfected by swabbing with 70% alcohol before working. The feed shoe, mixing equipment and containers were previously sterilized by dry heat. Tablets were produced at a constant (slow) rate of 42 per minute.

The lower punch holder was fitted with a quartz force ring (model 202A, PCB Piezotronics Inc.) to monitor the level of compression acting on the contaminated excipients. The force ring was calibrated against a cell traceable to National Physical Laboratory standards, using a Dartec 100kN M2501 universal testing

machine (Dartec Ltd.). Figure 4.1 shows the calibration curve of voltage output against compression force.

Figure 4.2 is a schematic representation of the force-monitoring system used. The force ring was activated by an external power supply, and the voltage output fed through an integrated circuit piezoelectric amplifier (model 484B, PCB Piezoelectronics Inc.) to an oscilloscope with a data capture and storage facility (Nicolet, model 3091). The system was set to observe data points between the start of compression and the end of the ejection phase. A standard 555 timer chip was used, with the resolution of the time frame set by the frequency output from an RS 2MHz function generator (RS Components Ltd.).

#### 4.1.3 The relationship between compression force and microbial kill

##### Method

Contaminated, directly-compressible excipients, prepared as described in section 3.1.3 above, were used to manufacture tablets at a range of compression forces, up to about 28 kN (323 MPa, or  $\text{MNm}^{-2}$ ).

The formulations used consisted of contaminated maize starch, Avicel PH101 or lactose, with magnesium stearate 1% w/w as lubricant.

Viable counts were determined as before (section 2.3), using two weighed samples consisting of three whole tablets. These were

Figure 4.1 Force ring voltage output as a function of  
compression force

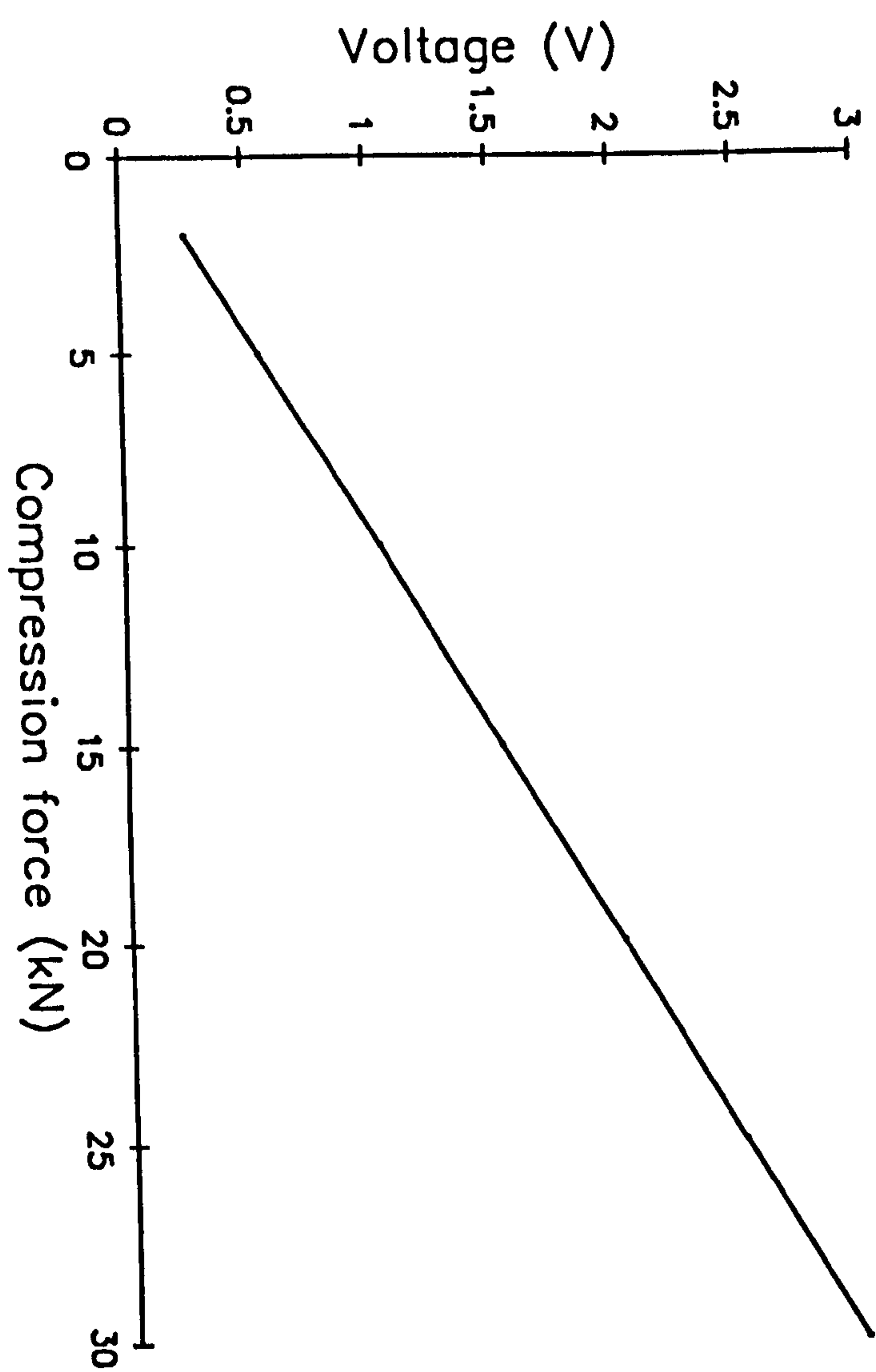
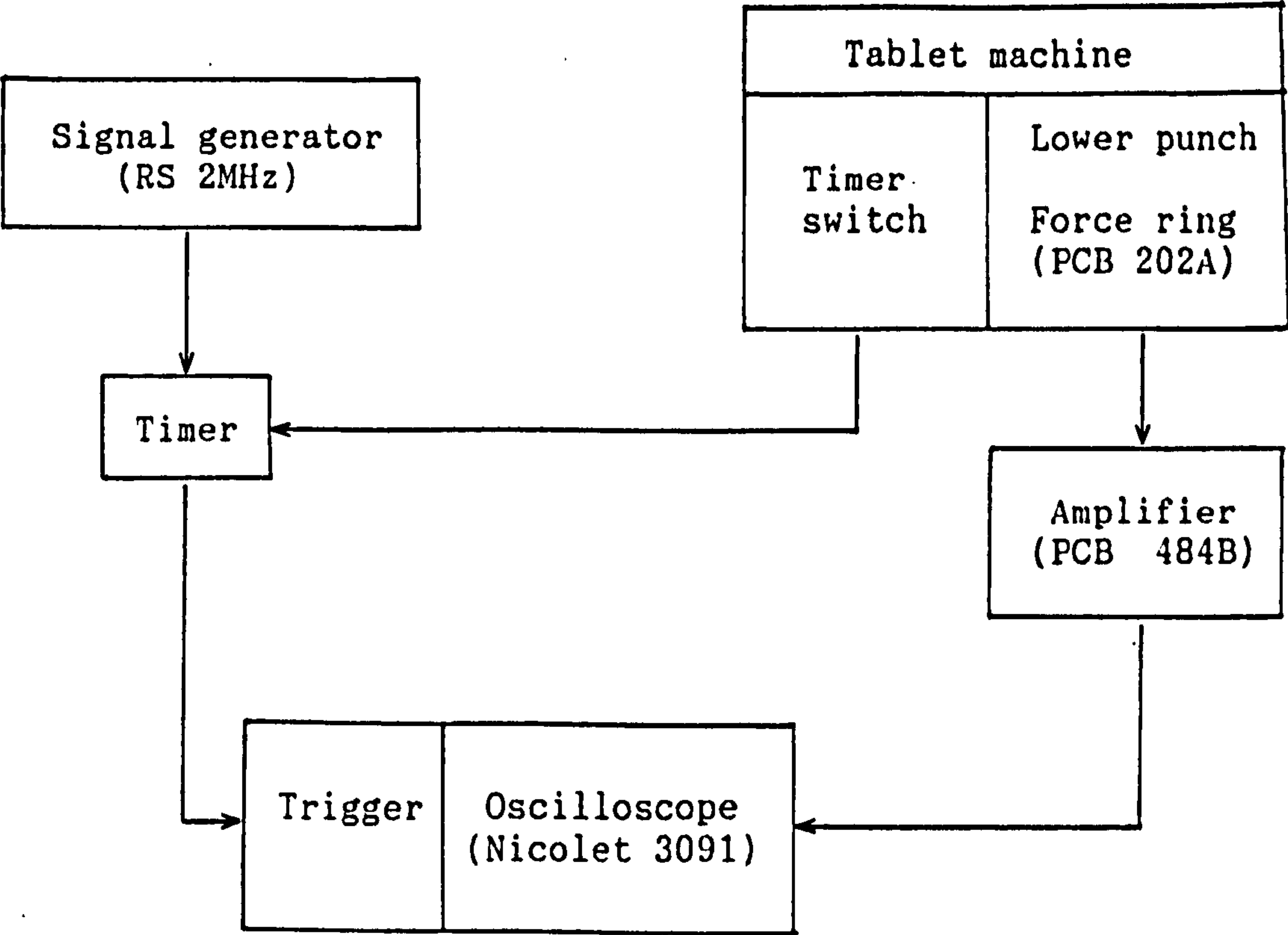




Figure 4.2 Schematic diagram of system to capture lower punch compaction data



Timer unit range

Minimum timeframe: 125 - 350 ms

Maximum timeframe: 275 - 2600 ms

compared with viable counts of bacteria in the uncompressed material, and graphs were plotted of log percentage inactivation against the compression force.

### Results

Table 4.1 lists the results of viable counts carried of the contaminated materials, before and after tableting. Figures 4.3 and 4.4 show the pressure/survival plots. Bacterial inactivation on tableting depended on the applied pressure, and varied according to the excipients used and the type of bacteria present. Satisfactory tablets, with contamination levels in the order of  $10^6$ - $10^8$  cfu/gram, could be prepared using compression forces in the range of 0.25-5 kN, depending on the formulation.

### 4.2 DISCUSSION OF RESULTS

Tablets were produced from contaminated starches, Avicel PH101 and lactose at a range of compression settings. A uniform, slow speed of tablet production was used, and the compaction pressure was monitored by a force ring mounted below the lower punch. Figures 4.3 and 4.4 show pressure/survival plots of log per cent survivors as a function of compaction pressure, for different types of contaminated excipient.

The results indicate that, as found by Fassihi (1978), Chesworth (1980) and Plumpton (1982), tablet compaction leads to a substantial loss of viability for contaminating bacteria. In this work, a three log unit reduction (i.e. 99.9% kill) was achievable for both Ent. cloacae and Staph. aureus.

Table 4.1 Compression force vs survival

(i) Staphylococcus aureus / lactose (170 mesh)

Compression force, kN	Viable count cfu/g	% survivors	log (% survivors)
0	$1.84 \times 10^8$	100	2
5.014	$9.67 \times 10^7$	52.43	1.720
9.613	$6.37 \times 10^7$	34.54	1.538
11.687	$5.38 \times 10^7$	29.30	1.460
14.718	$3.94 \times 10^7$	21.44	1.330
19.831	$2.85 \times 10^7$	15.45	1.189
25.630	$1.98 \times 10^7$	10.73	1.031
27.480	$1.92 \times 10^7$	10.41	1.017

(ii) Enterobacter cloacae / lactose (170 mesh)

Compression force, kN	Viable count cfu/g	% survivors	log (% survivors)
0	$2.33 \times 10^7$	100	2
2.442	$1.08 \times 10^7$	46.50	1.660
7.026	$5.07 \times 10^6$	21.73	1.337
7.947	$3.54 \times 10^6$	15.17	1.181
12.581	$2.87 \times 10^6$	12.32	1.091
17.340	$1.09 \times 10^6$	4.66	0.669
25.150	$5.13 \times 10^5$	2.10	0.340
27.494	$2.44 \times 10^5$	1.04	0.019

(iii) Staphylococcus aureus / maize starch

Compression force, kN	Viable count cfu/g	% survivors	log (% survivors)
0	$7.80 \times 10^8$	100	2
3.169	$7.00 \times 10^7$	8.97	0.953
5.338	$5.30 \times 10^7$	6.79	0.832
10.400	$1.30 \times 10^7$	1.67	0.222
14.271	$6.10 \times 10^6$	0.78	-0.107
17.018	$4.35 \times 10^6$	0.56	-0.254
20.976	$1.70 \times 10^6$	0.22	-0.662
24.629	$1.24 \times 10^6$	0.16	-0.799



(iv) Enterobacter cloacae / maize starch

Compression force, kN	Viable count cfu/g	% survivors	log (% survivors)
0	$1.36 \times 10^8$	100	2
0.426	$1.90 \times 10^7$	14.03	1.146
2.920	$6.14 \times 10^6$	4.51	0.655
4.673	$3.93 \times 10^6$	2.89	0.460
7.670	$2.19 \times 10^6$	1.61	0.206
9.853	$5.91 \times 10^5$	0.43	-0.362
20.499	$2.00 \times 10^5$	0.15	-0.832
22.702	$1.59 \times 10^5$	0.12	-0.930

(v) Staphylococcus aureus / Avicel PH101

Compression force, kN	Viable count cfu/g	% survivors	log (% survivors)
0	$9.58 \times 10^8$	100	2
1.057	$3.11 \times 10^8$	32.45	1.511
3.838	$1.44 \times 10^8$	15.07	1.178
9.222	$3.56 \times 10^7$	3.72	0.570
16.238	$2.82 \times 10^7$	2.94	0.468
19.753	$2.17 \times 10^7$	2.26	0.354
26.532	$1.69 \times 10^7$	1.76	0.246

Figure 4.3 Pressure/survival plot for Ent. cloacae tableted in maize starch or lactose

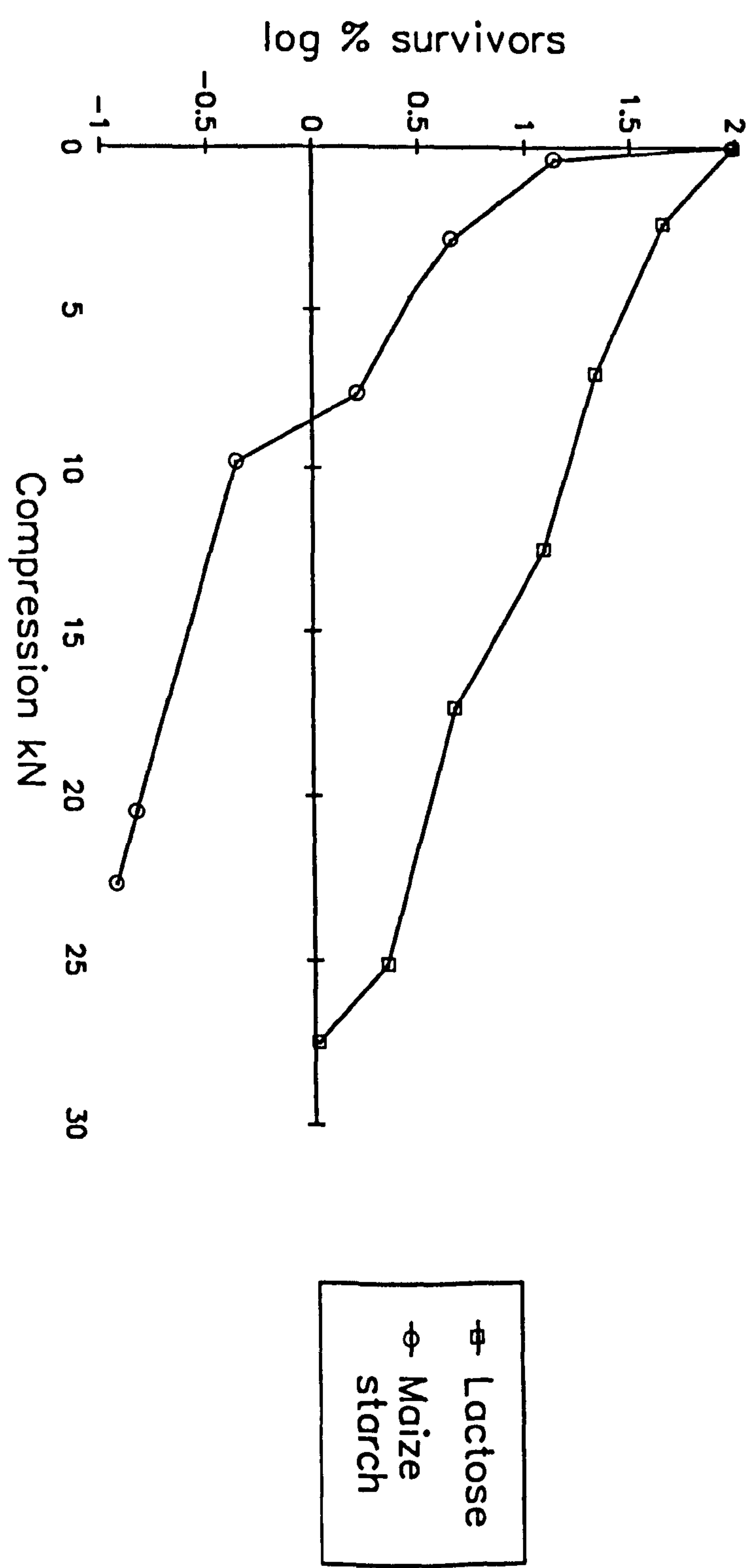
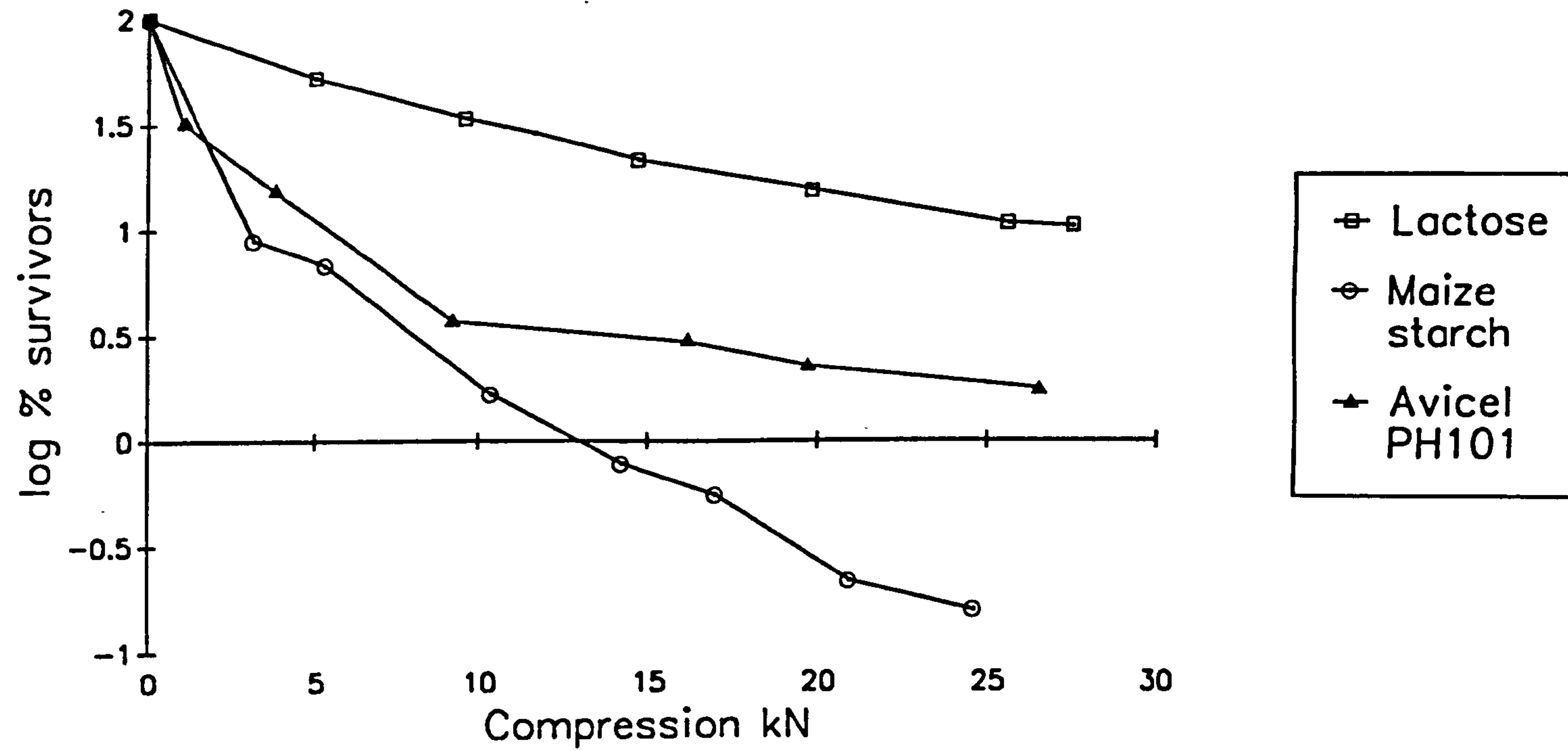


Figure 4.4 Pressure/survival plot for Staph. aureus tableted in maize starch, lactose, or Avicel PH101





It would seem that the smaller, coccoid cells of Staph. aureus were more resistant to the harmful effects of tableting than the larger, Ent. cloacae rods. It would appear that during tableting, organisms presenting a smaller surface area have a better chance of survival. These findings are in agreement with those of Plumpton (1982) and Plumpton et al (1986b).

Overall, the survival of bacteria during tableting was inversely related to the compaction pressure applied. For lactose, this relationship appears to be a direct inverse proportionality, with a correlation coefficient for linearity of -0.99 for both organisms. For maize starch and Avicel PH101 however, the slope of the pressure/survival plots was greatest at low compaction pressures, and decreased sharply at higher compaction pressures.

The three excipients have different mechanisms of consolidation on compaction. Lactose monohydrate is a brittle crystalline material, and tends to consolidate by fracture initiated at the early stages of compression (Hersey et al, 1973). As the applied force is increased, more of the material fractures and rearranges to fill available void spaces. In contaminated lactose granules, the inverse proportionality of bacterial survival to applied pressure probably results from increasing the areas of interparticulate contact on fracturing; the micro-organisms may then be inactivated by shearing forces, as described by Plumpton et al (1986a).

Maize starch is particularly prone to plastic flow, and less prone to plastic recovery than other starches (Paronen and Juslin,

1983). According to Hiestand et al (1977), plastic deformation results in localized shear flow, which under a compression load would lead to greater interparticulate contact, and enhance mechanical disruption of microbial contaminants. In the early stages of compression, maize starch deforms plastically, accompanied by particle rearrangement (Paronen and Juslin, 1983). At low compaction pressures, more extensive kill was observed in starch than lactose. As the compaction pressure is increased, densification of the starch compact hinders particle rearrangement, and this explains the relatively smaller rise in the extent of bacterial kill in starch at higher compaction pressures.

The pressure/survival plot obtained from compressions of Avicel PH101 contaminated with Staph. aureus initially follows the same pattern as for maize starch. However, at compression forces exceeding about 8 kN (corresponding to 92 MPa for the punches used), a linear relationship developed, with a correlation coefficient for linearity of -0.99, and a slope similar to that obtained for Staph. aureus tableted in lactose. The plastically-deforming nature of Avicel PH101 has been confirmed by David and Augsberger (1977), but it was reported by Sixsmith (1982) that Avicel PH101 exceeds its elastic limit after about 80 MPa, and at higher pressures consolidates chiefly by brittle fracture. This explains the change in the extent of bacterial kill in Avicel PH101 tablets as the compression forces were increased, and provides strong evidence that shear flow is the chief mechanism of



kill, since inactivation was substantially reduced once the elastic limit was exceeded.

These results suggest that compression of plastically-deforming materials causes more bacterial inactivation than fracturing materials, especially at low pressures. This conflicts with the conclusions of Fassihi and Parker (1987), who found that greater microbial inactivation occurred in brittle materials. No general relationship between levels of kill and compaction mechanisms were reported by Plumpton et al (1986a). The disparity between results may be explained by the use of a lubricant to facilitate tableting. Fassihi and Parker (1987) did not incorporate a lubricant into the granules when tableting the brittle materials, whereas a lubricant was added to the granules of the plastically-deforming material. In the investigations of Plumpton et al (1986a) and those reported here, a lubricant was mixed with all of the granule samples. It is likely that interparticulate friction was a significant factor in determining the degree of inactivation observed in brittle materials by Fassihi and Parker (1987), but that this effect was masked and shearing reduced by lubricants within the systems used by Plumpton et al (1986a), and in these studies.

It would seem that under controlled conditions, micro-organisms have a potential use as biological monitors, to determine the levels and types of forces acting within a compressed formulation. This relationship was subsequently explored during extrusion processing, using contaminated raw materials.



## 5. INVESTIGATIONS OF EXTRUSION AND SPHERONISATION

## 5.1 METHODS AND RESULTS

### 5.1.1 Introduction

The factors affecting microbial inactivation during tablet compression have been the subject of debate, particularly the role of shearing forces (Fassihi and Parker, 1987; Plumpton et al, 1986a). In the studies that follow, the relative effects of compression and shear forces on bacterial viability were examined during the extrusion of a wet powder mass, a process used commercially in the pharmaceutical industry to produce spheroids for solid oral dosage forms.

Granule formulations for extrusion/spheronisation commonly use microcrystalline cellulose, which exhibits the specific plasticity required to form spheroids. Harrison et al (1987) studied controlled extrusion through a single die, using a formulation of microcrystalline cellulose (Avicel PH101), lactose and water in the ratio of 5:5:6. This formulation was said to closely resemble "a practical pharmaceutical formulation", and the same materials and proportions were used for these studies.

### 5.1.2 Extrusion of contaminated wet granules

The growth of organisms was studied in a wet powder mass contaminated with Ent. cloacae. Further investigations followed survival during extrusion and spheronisation.

5.1.2.1 Survival/growth of Ent. cloacae in a wet powder mass, prepared with peptone buffer or distilled water

Method

(i) A contaminated wet powder mass was prepared from Avicel PH101 (60g), lactose 300 mesh (60g). The aqueous phase was a working suspension of peptone buffer (72ml), adjusted as described in section 2.6.3 to contain about  $10^7$  organisms per ml.

(ii) A second batch of wet powder mass was prepared as above, using as the aqueous phase a working suspension of Ent. cloacae (about  $10^7$  organisms per ml) in sterile distilled water. The working suspension was prepared by harvesting and centrifuging the bacterial cell suspensions in peptone buffer as described in 2.6.3, and diluting to produce the final working suspension in sterile distilled water.

Each batch was prepared by mixing the dry ingredients, and gradually adding the aqueous phase. A planetary mixer (Kenwood) was used, with stainless steel bowl and blade previously heat-sterilized. The wet mass was stored at 25°C for 8 hours in sterile screw-capped jars. Three weighed samples (1g) were withdrawn from each batch at hourly intervals, and the viable counts determined.

Results

The viable counts for each batch of wet mass are shown on table 5.1. Graphs of log(viable count/g) were plotted against storage time and are shown in figure 5.1. Although growth in the presence of peptone buffer was rapid and marked, there was also clearly



Table 5.1 Growth of Ent. cloacae in a wet powder mass at 25°C.

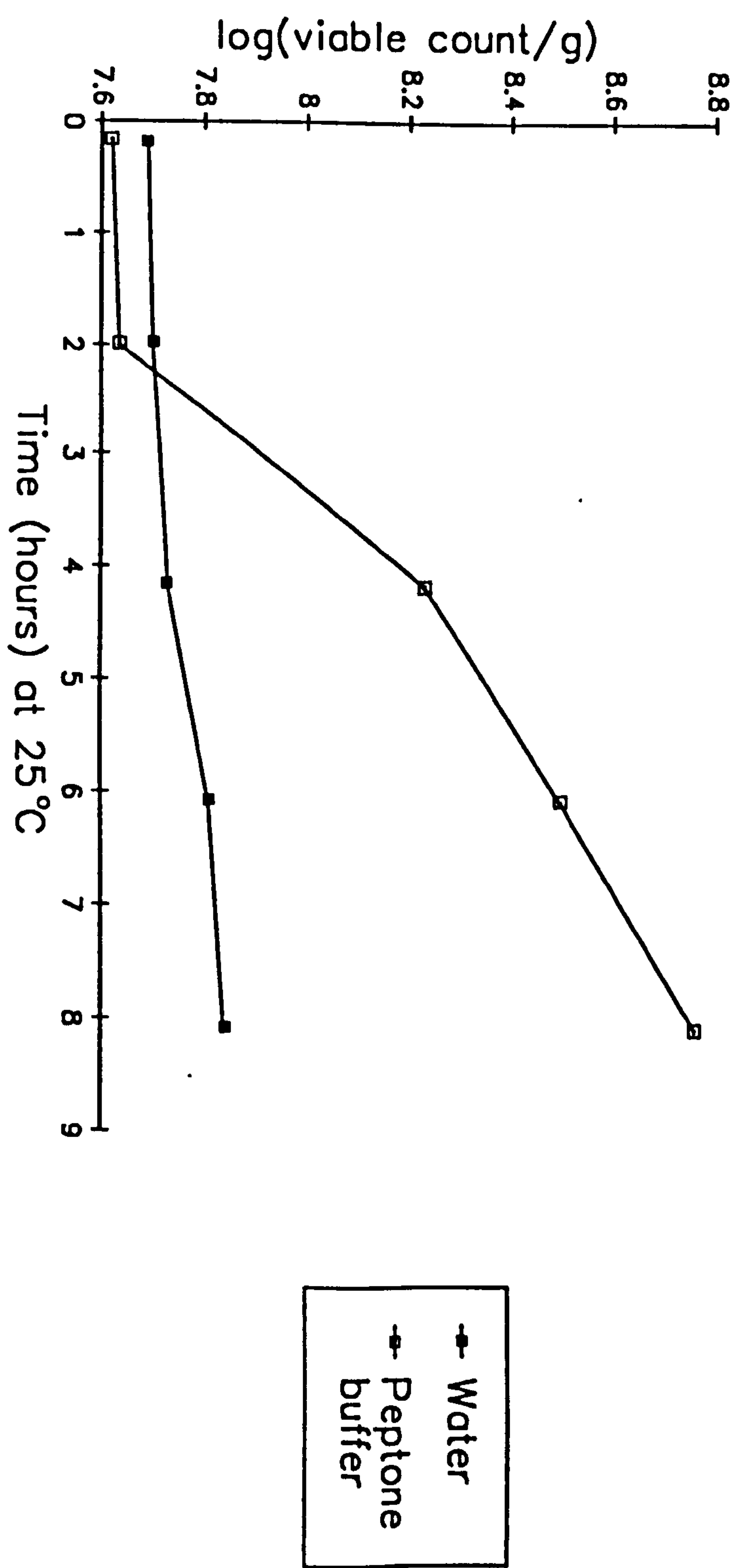
(i) Wet powder mass contaminated with peptone buffer:

Storage time (hr. min.)	Viable count (cfu/g)	Growth factor	log viable count (cfu/g)
0.10	$4.17 \times 10^7$	1.00	7.62
2.00	$4.27 \times 10^7$	1.02	7.63
4.10	$1.70 \times 10^8$	3.07	8.23
6.05	$3.09 \times 10^8$	6.41	8.49
8.05	$5.75 \times 10^8$	12.78	8.76

(ii) Wet powder mass contaminated with distilled water:

Storage time (hr. min.)	Viable count (cfu/g)	Growth factor	log viable count (cfu/g)
0.10	$4.90 \times 10^7$	1.00	7.69
2.00	$5.01 \times 10^7$	1.02	7.70
4.10	$5.37 \times 10^7$	1.10	7.73
6.05	$6.46 \times 10^7$	1.32	7.81
8.05	$6.92 \times 10^7$	1.41	7.84

Figure 5.1 Growth of Ent. cloacae in a wet powder mass  
at 25°C



significant growth in the system based on water (  $P < 0.001$  at 8.05 hours). The results showed that Ent. cloacae could survive and grow in the stored wet powder mass, although the rate of growth was greatly enhanced where peptone was present as a nutrient source.

#### 5.1.2.2 The survival of Ent. cloacae during ram extrusion

According to Harrison (1982) the forces acting on a powder during extrusion depend upon the stage of the process and the position of the material relative to the barrel and die.

Ram extrusion of contaminated wet powder masses was carried out through a single die, to determine whether the survival of organisms was related to the magnitude of forces at different stages of extrusion.

A wet powder mass was prepared as described above (5.1.2.1), using Avicel PH101 (125g), lactose 300 mesh (125g) and a working suspension (based on peptone buffer) of Ent. cloacae as the aqueous phase (150ml). The initial viable count in the wet powder mass was  $7.0 \times 10^7$  organisms per gram. The wet mass was stored overnight (14 hours) at  $25^{\circ}\text{C}$  in sealed, double thickness plastic bags. The viable count following storage was  $8.1 \times 10^8$  organisms per gram.

For each extrusion, 50g of the contaminated wet mass was hand-packed into the steel extruder barrel, which was about 20cm long, with an internal radius of 1.27cm. A single die of length 3mm and



radius 0.5mm (i.e. a length to radius ratio of 6) was situated centrally at one end of the barrel. The barrel was mounted onto a C-piece under a piston attached to the crosshead of a mechanical press (Instron Ltd., Floor Model TT), previously calibrated using a load cell. Figure 5.2 shows the extruder barrel arrangement. Outputs from the load cell and from a displacement transducer (Sangamo Transducers) were monitored as force-displacement plots on an X-Y recorder (Gould series 60000).

Extrusions were carried out at a constant ram speed of 10cm/minute, and the extrudate was sampled during steady-state flow. On completion of the compression cycle, the extruder barrel was dismantled to sample unextruded material which had been subjected to the high compression forces within the barrel during forced-flow. To collect samples of unextruded material during steady state flow, the compression cycle was interrupted and the barrel dismantled. The contaminated material at the barrel wall and the centre of the barrel was sampled. Viable counts of three 1g samples were determined. Plates 4 and 5 show scanning electron micrographs of the extrudate in cross section.

### Results

Figure 5.3 shows the force/displacement profiles for the process, and the viable count results are summarized in figure 5.4 and table 5.2.

The results of viable counts from samples taken at different stages of ram extrusion, all differed significantly at the 95% confidence level. There was a clear difference in bacterial

Figure 5.2 The ram extruder barrel arrangement

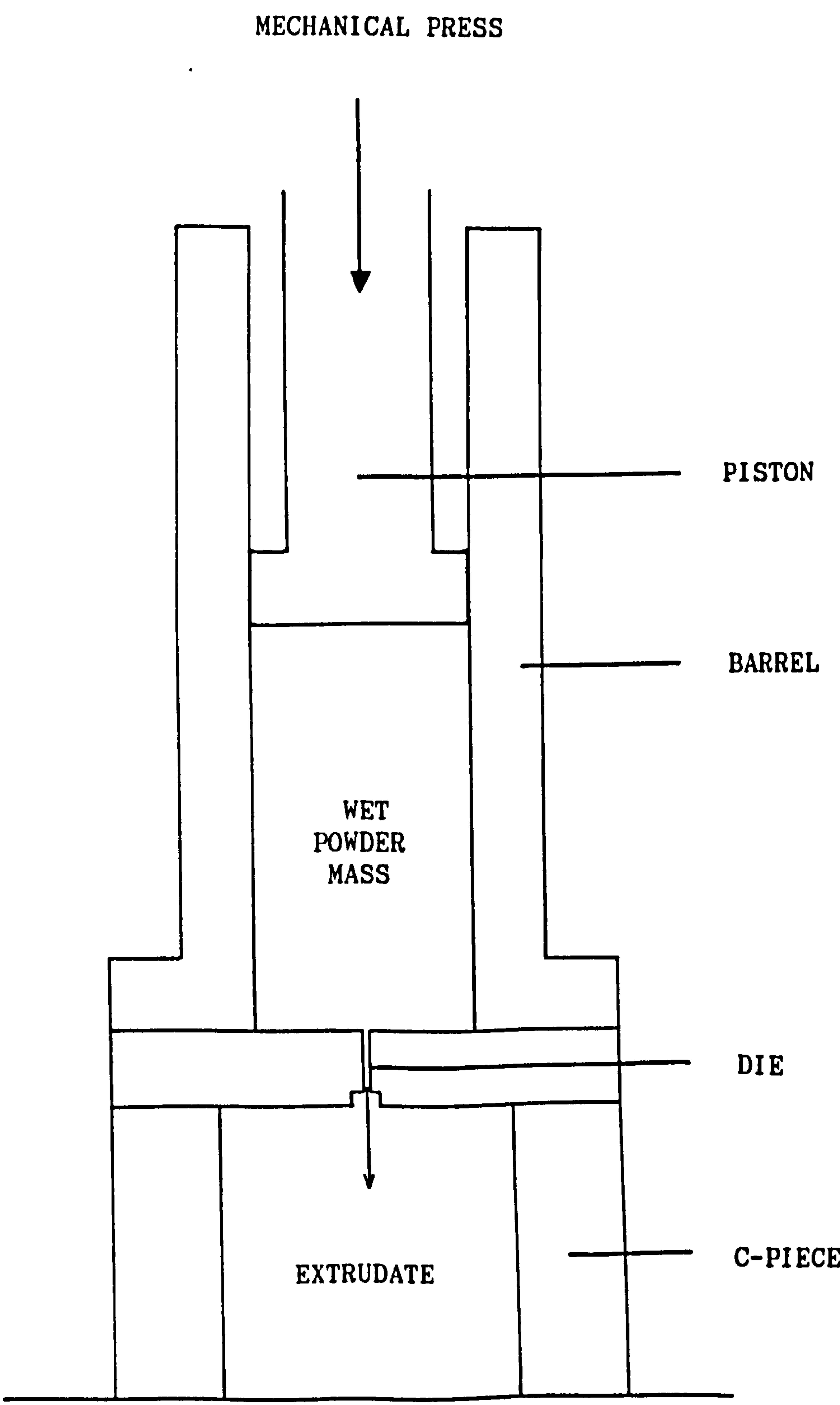




Plate 4. Scanning electron micrographs of extrudate

(a) Extrudate in cross-section (x55)



(b) Detail of plate 4(a), showing cross-section and material compressed against the die wall (x220)

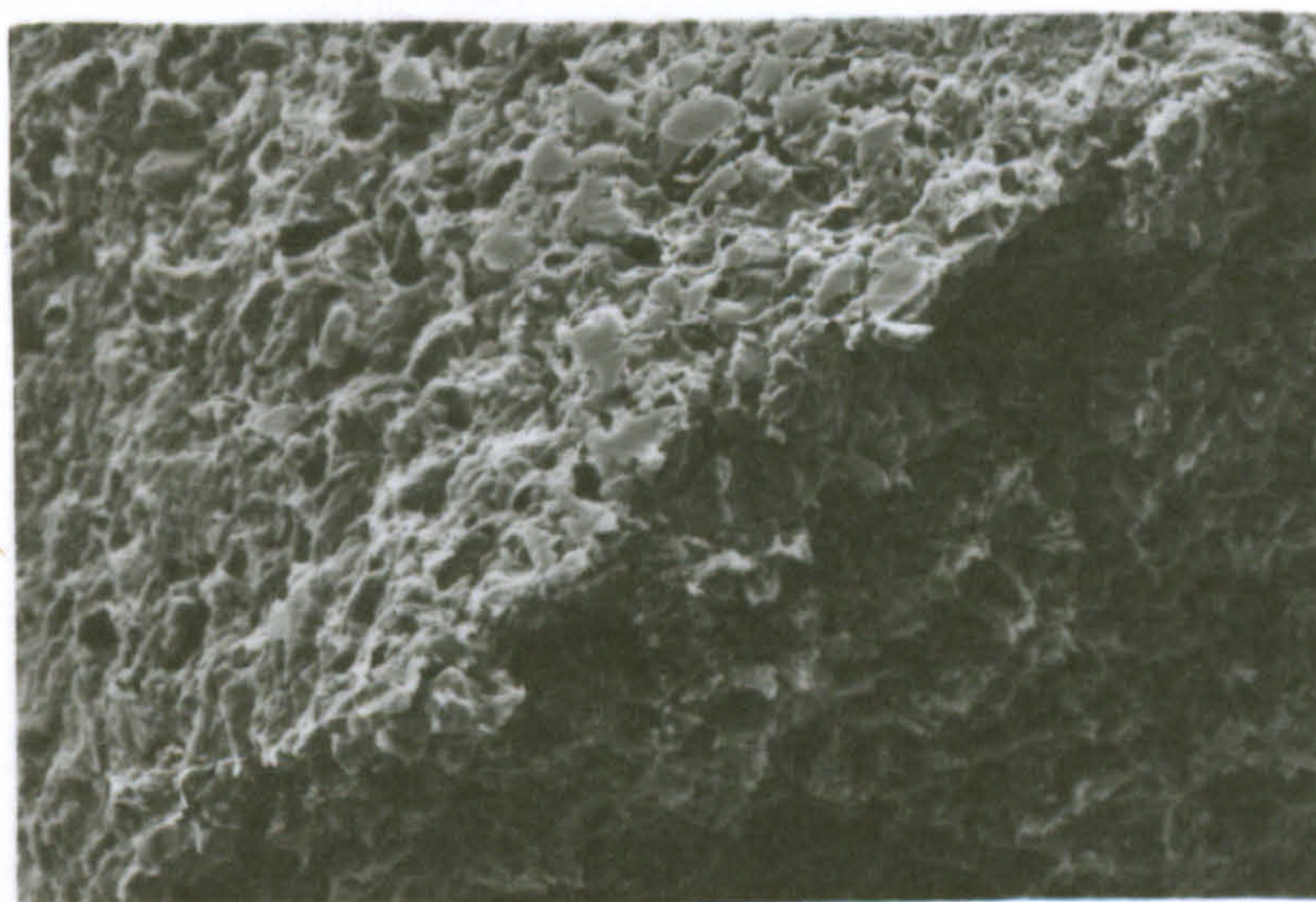
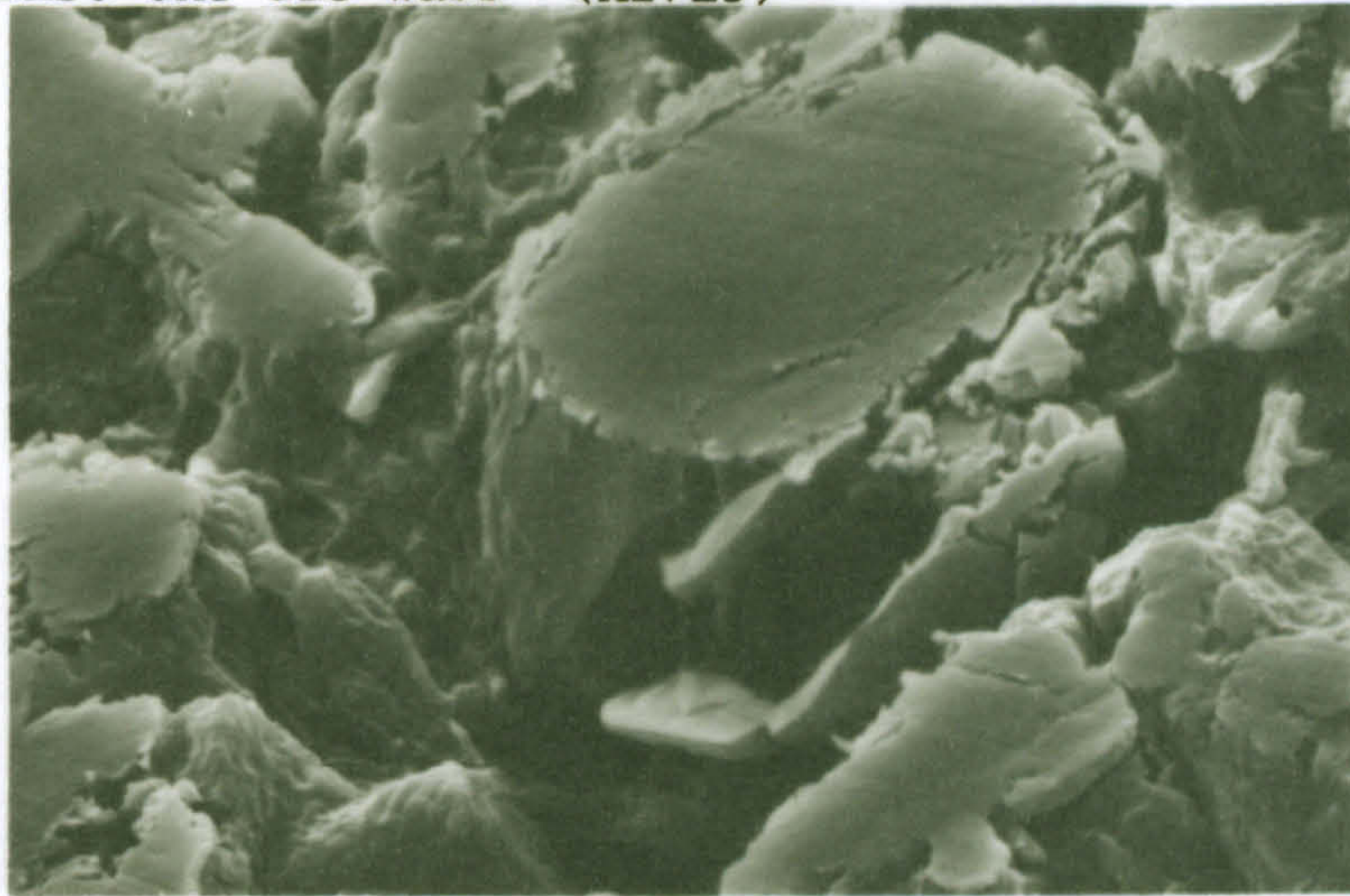




Plate 5. Scanning electron micrographs of extrudate

- (a) Detail of plate 4(a), showing material compressed against the die wall (xl725)



- (b) Detail of plate 4(a), showing extrudate in cross-section (xl725)

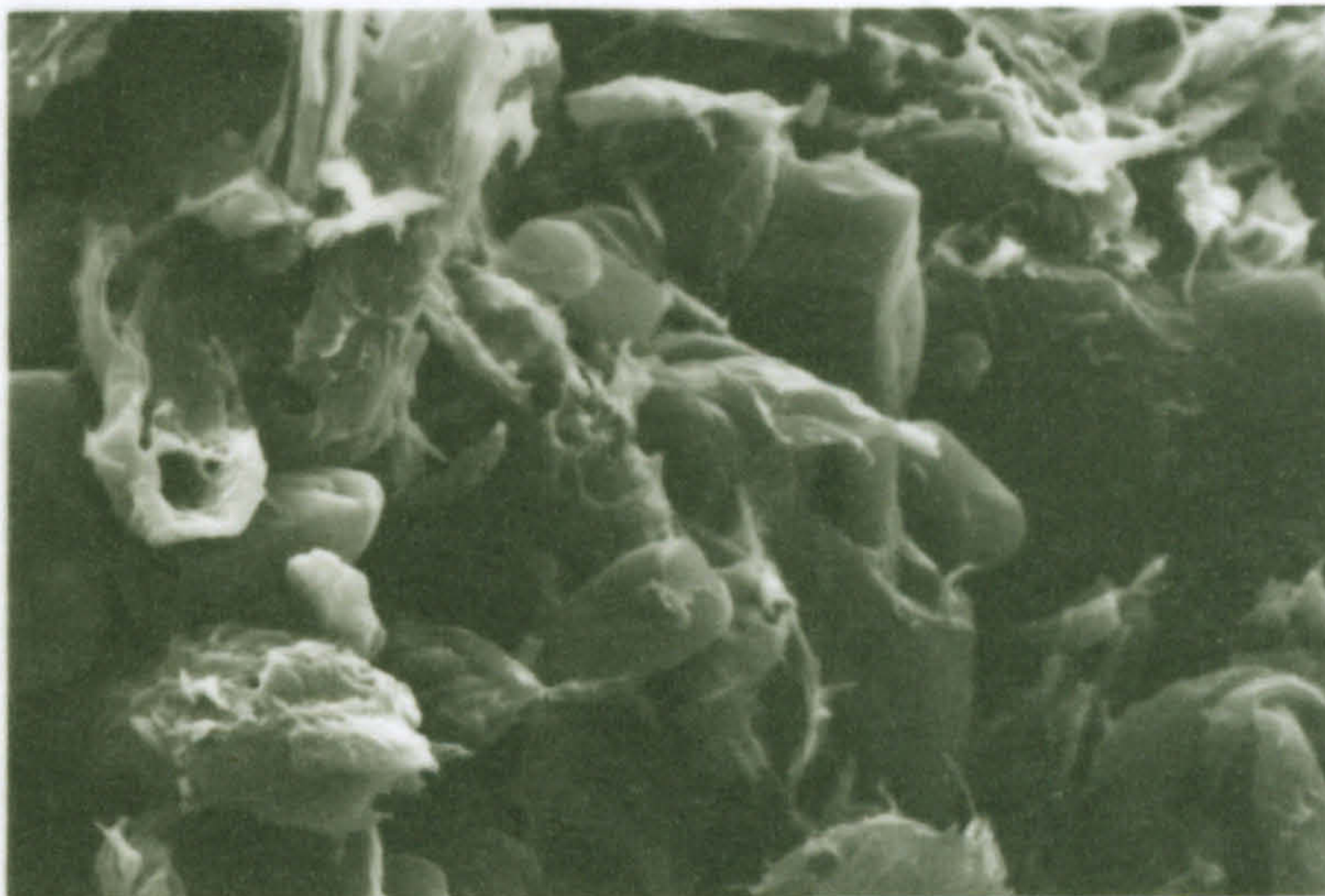




Figure 5.3 Force-displacement profiles for extrusions at 10cm/minute

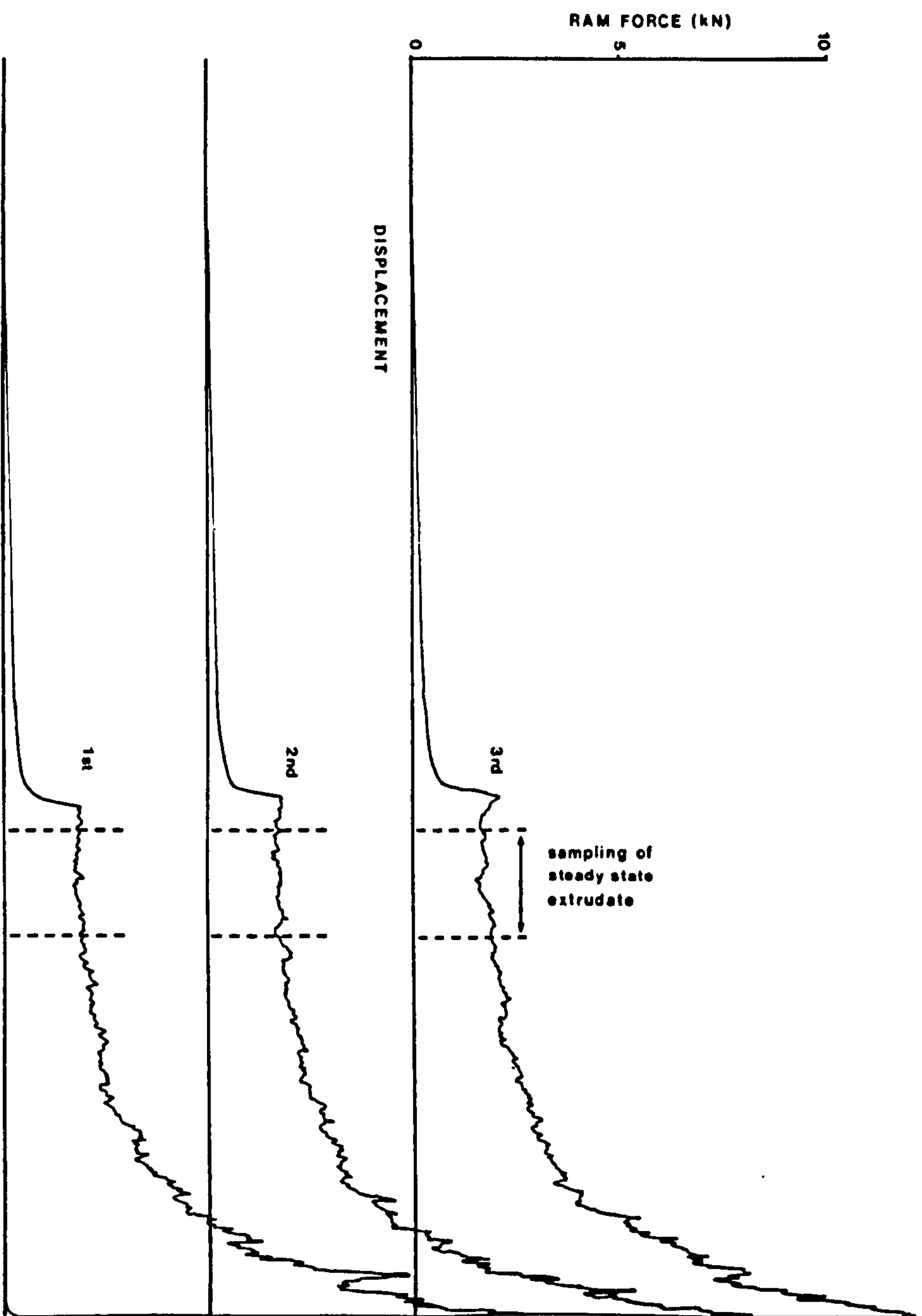
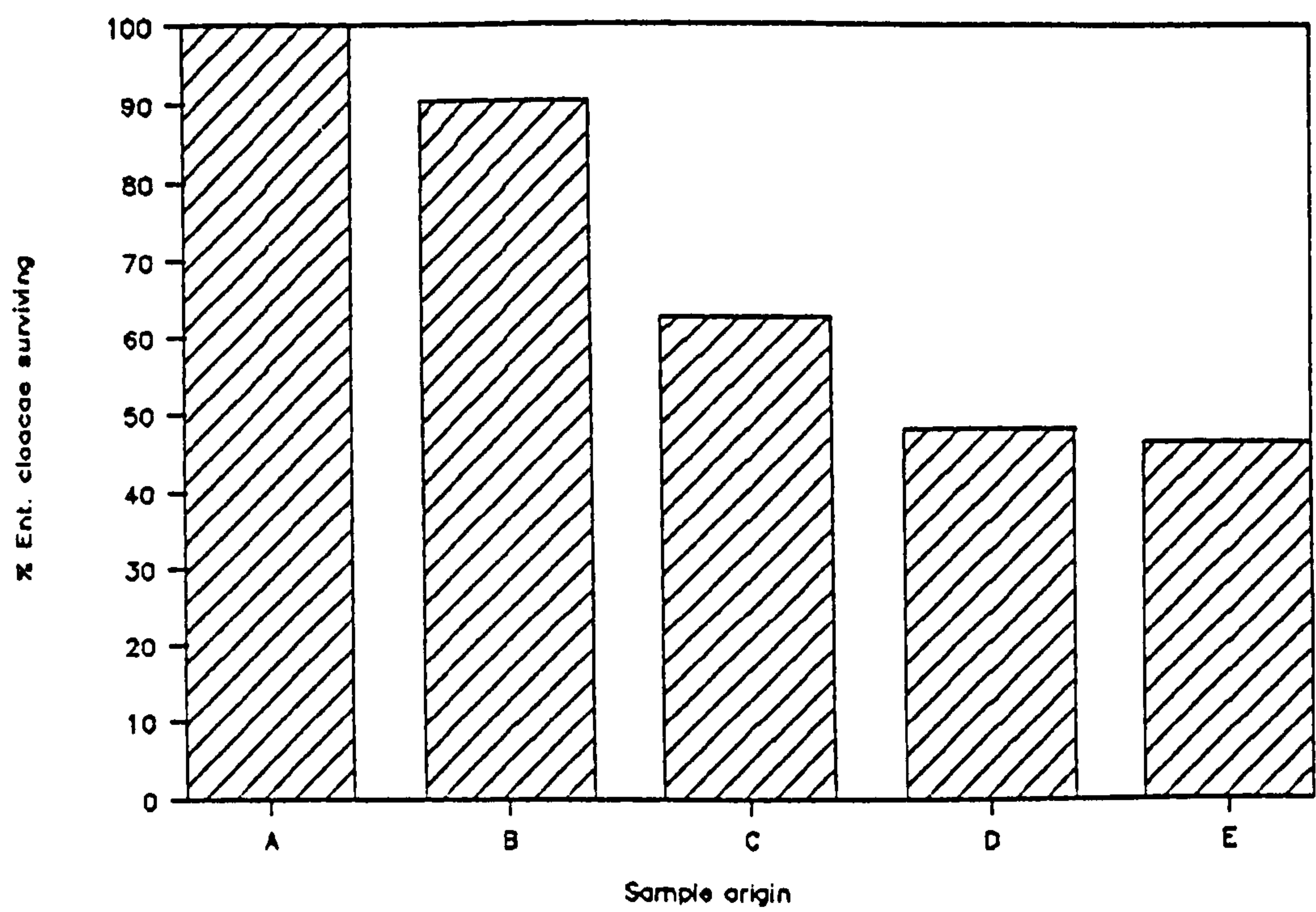


Figure 5.4 Chart showing the levels of Ent. cloacae surviving ram extrusion of a contaminated wet powder mass



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Sample origin

- A    unprocessed material
  - B    barrel contents during steady-state flow
  - C    material at barrel wall during steady-state flow
  - D    barrel contents during forced-flow
  - E    steady-state extrudate
-



Table 5.2    The effect of the stage of ram extrusion on the survival of Ent. cloacae introduced via the aqueous phase.

Ram speed = 10 cm/minute  
Length to radius ratio = 6

Sample origin	Viable count/g	% Survivors
A    unprocessed material	8.55x10 <sup>8</sup>	100
B    barrel contents during steady-state flow	7.73x10 <sup>8</sup>	90.43
C    material at barrel wall during steady-state flow	5.37x10 <sup>8</sup>	62.82
D    barrel contents during forced-flow	4.12x10 <sup>8</sup>	48.20
E    steady-state extrudate	3.98x10 <sup>8</sup>	46.56

Viable counts from processed samples compared with unprocessed material:

		% cv	$t = \frac{\bar{x} - Ax}{s / n}$	P
A	1.04x10 <sup>7</sup>	1.24	-	-
B	3.36x10 <sup>7</sup>	4.35	3.478	0.02-0.05
C	2.60x10 <sup>7</sup>	4.88	9.310	< 0.001
D	1.65x10 <sup>7</sup>	4.07	12.082	< 0.001
E	1.38x10 <sup>7</sup>	3.47	13.072	< 0.001

survival when viable counts of the barrel contents (unextruded) were compared with counts of extrudate sampled during steady-state flow. During steady state flow within the extruder barrel, bacteria present in material moving at the barrel wall showed a higher rate of kill than those in material moving centrally within the barrel. Inactivation was greatest in the extruded material, and material which had undergone forced-flow within the barrel, where compression forces of up to 12 kN were recorded.

5.1.2.3 The survival of Ent. cloacae in the extrudate,  
according to the route of contamination and the  
ram speed used

Extrusion was carried out at different ram speeds, using batches of wet powder mass contaminated Ent. cloacae.

Method

The granules were formulated as before (see 5.1.2.1 above), but the method of contamination varied thus:-

(i) Avicel PH101 (160g); lactose 300 mesh (160g), aqueous phase peptone buffer (192ml) containing Ent. cloacae  $1.8 \times 10^8$  organisms per ml;

(ii) Avicel PH101 (160g); sterile peptone buffer (192ml); lactose 300 mesh (160g) previously incubated with Ent. cloacae (prepared as described in section 3.1.3, except that the wet lactose granules were not dried after screening) and containing  $1.0 \times 10^9$  organisms per gram;

(iii) Lactose 300 mesh (160g); sterile peptone buffer (192ml);

Avicel PH101 (160g) previously incubated with Ent. cloacae (method of contamination as described in section 3.1.3, except that the wet Avicel PH101 granules were not dried after screening) and containing  $1.3 \times 10^9$  organisms per gram.

The mixed wet powder masses were stored at 25°C for 14 hours to allow the added moisture to equilibrate. Table 5.3 shows the viable counts of the contaminated wet powder masses prior to extrusion.

Ram extrusion was carried out on 50g quantities of material, again using a die with radius 0.5mm and length 3mm, at a constant ram speed of either 5cm/minute, or 40cm/minute. The steady-state extrudate was sampled, to determine whether varying the method of contamination or ram speed affected survival.

### Results

The results are summarized on table 5.4. Despite the higher mean load applied to initiate steady-state flow at a ram speed of 40cm/minute, there was no statistical difference in bacterial survival at the different ram speeds. At both ram speeds, the level of kill was slightly higher for extrudate prepared with contaminated peptone buffer granulating fluid, than for extrudate where the bacteria were grown in the excipient powders.



Table 5.3 Bacterial load of damp granules prior to extrusion

Mix	Source of contamination	Viable count/g
(i)	Peptone buffer	$1.98 \times 10^9$
(ii)	Lactose	$2.25 \times 10^9$
(iii)	Avicel PH101	$1.76 \times 10^9$

Table 5.4 The effect of varying route of inoculation and ram displacement speed on the survival of Ent.cloacae following extrusion

(i) Peptone buffer

Ram speed (cm/min)	Mean load (kg) for steady -state flow	Viable count (cfu/g)	% cv	Survivors %
0	0	$5.58 \times 10^9$	4.92	100
5	115	$1.70 \times 10^9$	3.01	30.47
40	298.3	$1.80 \times 10^9$	5.57	32.26

(ii) Lactose

Ram speed (cm/min)	Mean load (kg) for steady -state flow	Viable count (cfu/g)	% cv	Survivors %
0	0	$2.28 \times 10^{10}$	2.23	100
5	90	$1.00 \times 10^{10}$	2.11	43.74
40	265	$9.36 \times 10^9$	3.78	40.90

(iii) Avicel PH101

Ram speed (cm/min)	Mean load (kg) for steady -state flow	Viable count (cfu/g)	% cv	Survivors %
0	0	$7.14 \times 10^9$	3.85	100
5	80	$3.06 \times 10^9$	3.16	42.86
40	231.5	$2.80 \times 10^9$	4.39	39.20

#### 5.1.2.4 The effect of spheronisation on levels of contaminating bacteria

##### Method

Spheroids were produced from steady-state extrudate contaminated by different methods (as described above, section 5.1.2.3). A ram speed of 40cm/minute was used in this experiment. Batches of extrudate (200g) were transferred to an experimental-scale spheroniser with a 22.5cm diameter radial plate, and spun at 1000RPM for six minutes. During spheronisation, frictional forces from the rotating plate, and the plasticity of Avicel PH101, shape and densify the extruded material into spherical granules.

##### Results

Plate 6 illustrates the wet powder mass, extrudate and spheroids. Table 5.5 shows the viable counts of extrudate and spheroids, and the percentage inactivation of bacteria due to spheronisation. Statistical analysis of the viable count results indicated that bacterial inactivation on spheronisation was significantly greater where the extrudate was prepared with contaminated Avicel PH101.

The results of extrusion and spheronisation studies are summarized on table 5.6 and figure 5.5.

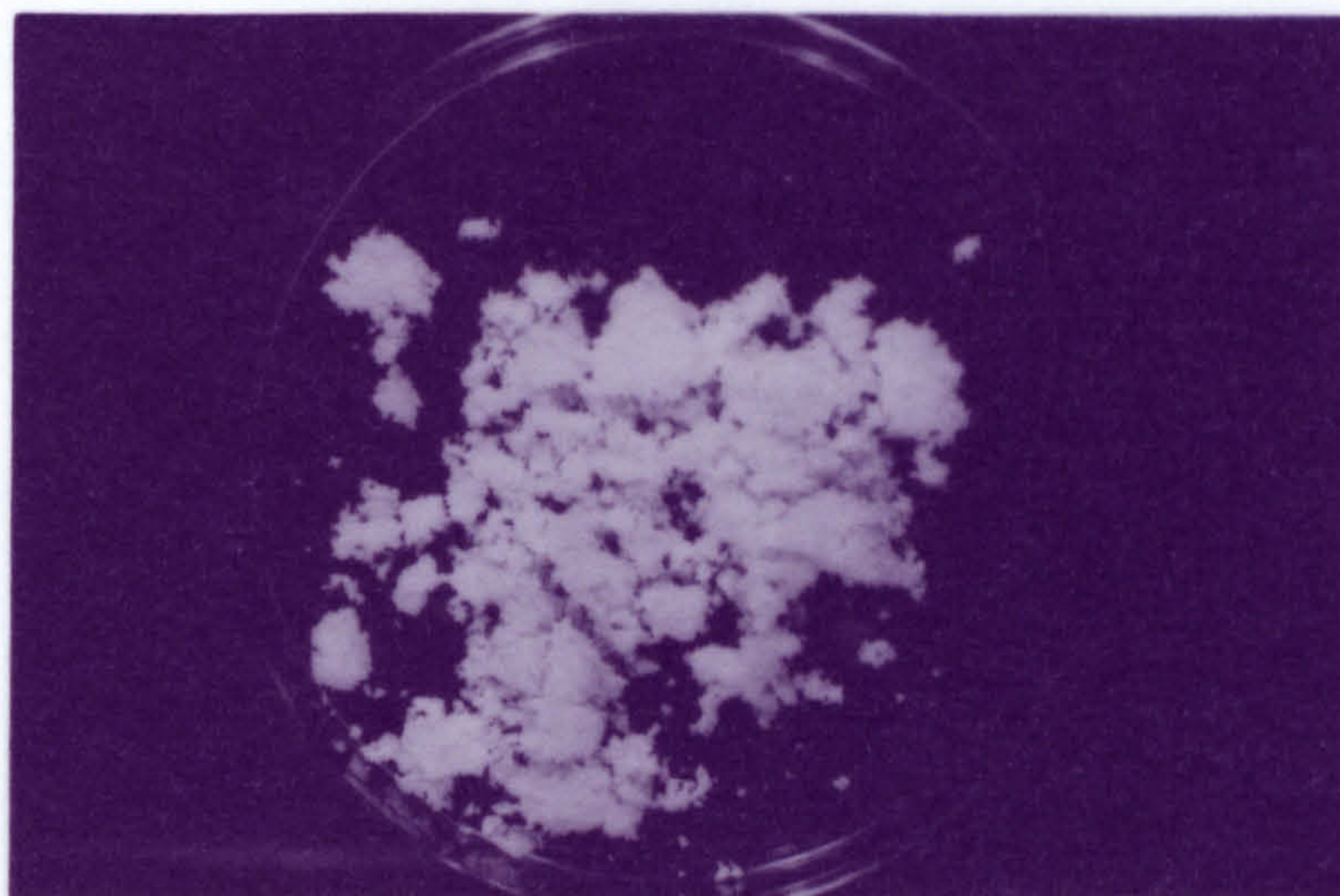
## 5.2 DISCUSSION OF RESULTS

Storage of moist extrusion mix at 25°C provided suitable conditions for the growth of Ent. cloacae. Some growth was observed in mixtures where water was used as the granulating fluid. Since earlier experiments demonstrated no growth of

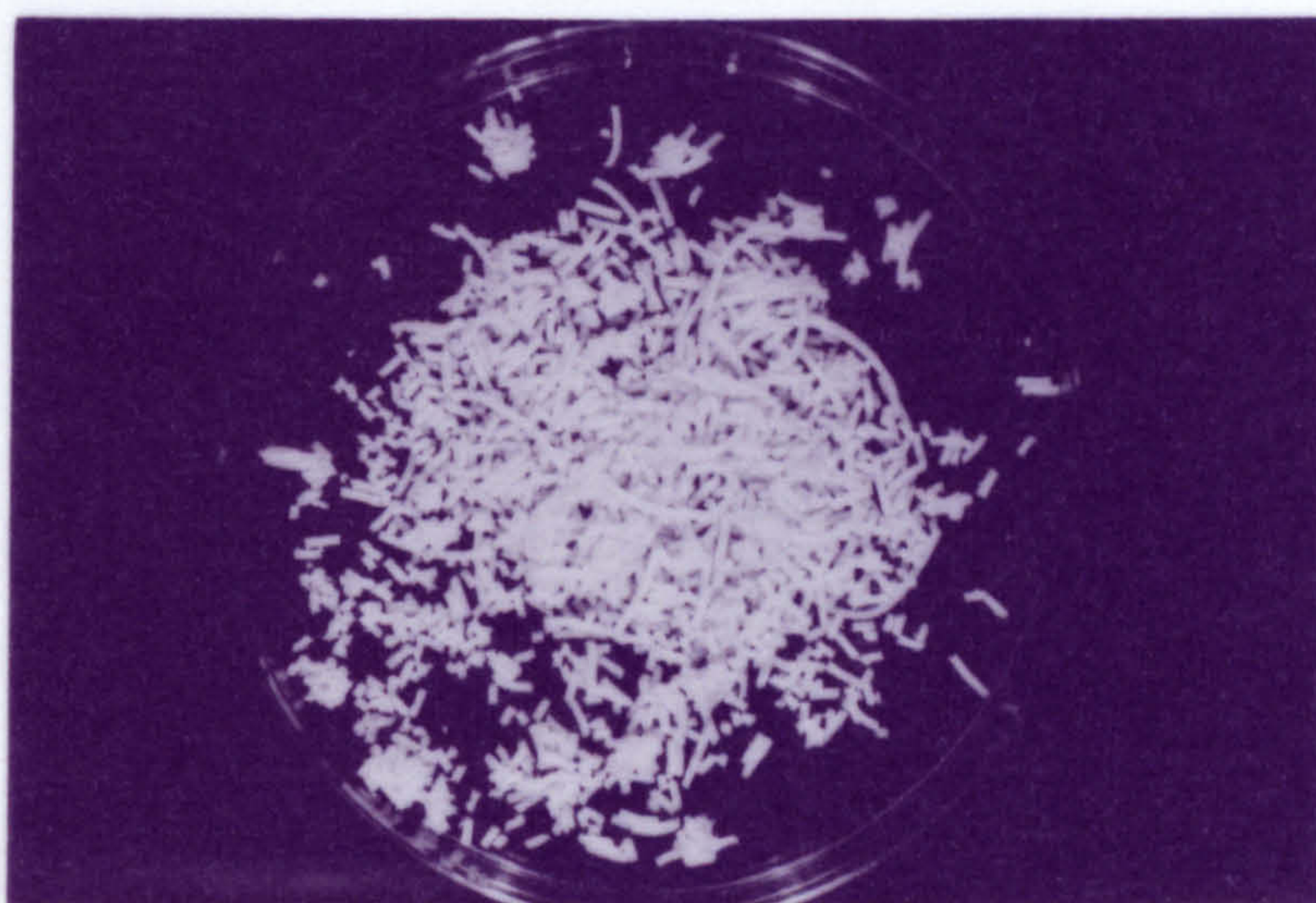


Plate 6. Wet powder mass processed by extrusion/spheronisation

(a) Wet powder mass



(b) 0.5mm diameter extrudate



(c) Spheroids

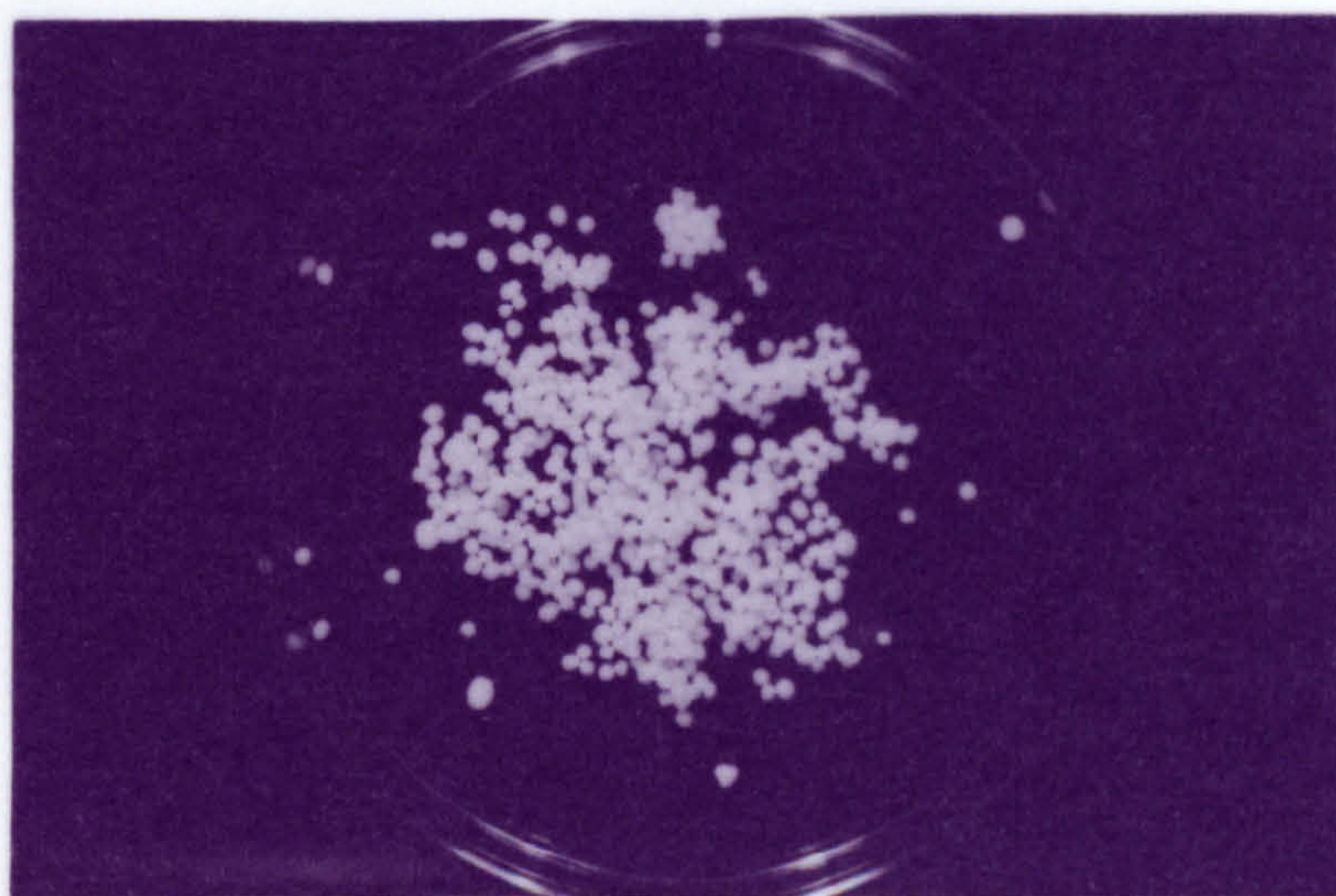




Table 5.5 The effect of method of contamination on the survival of Ent. cloacae following spheronisation

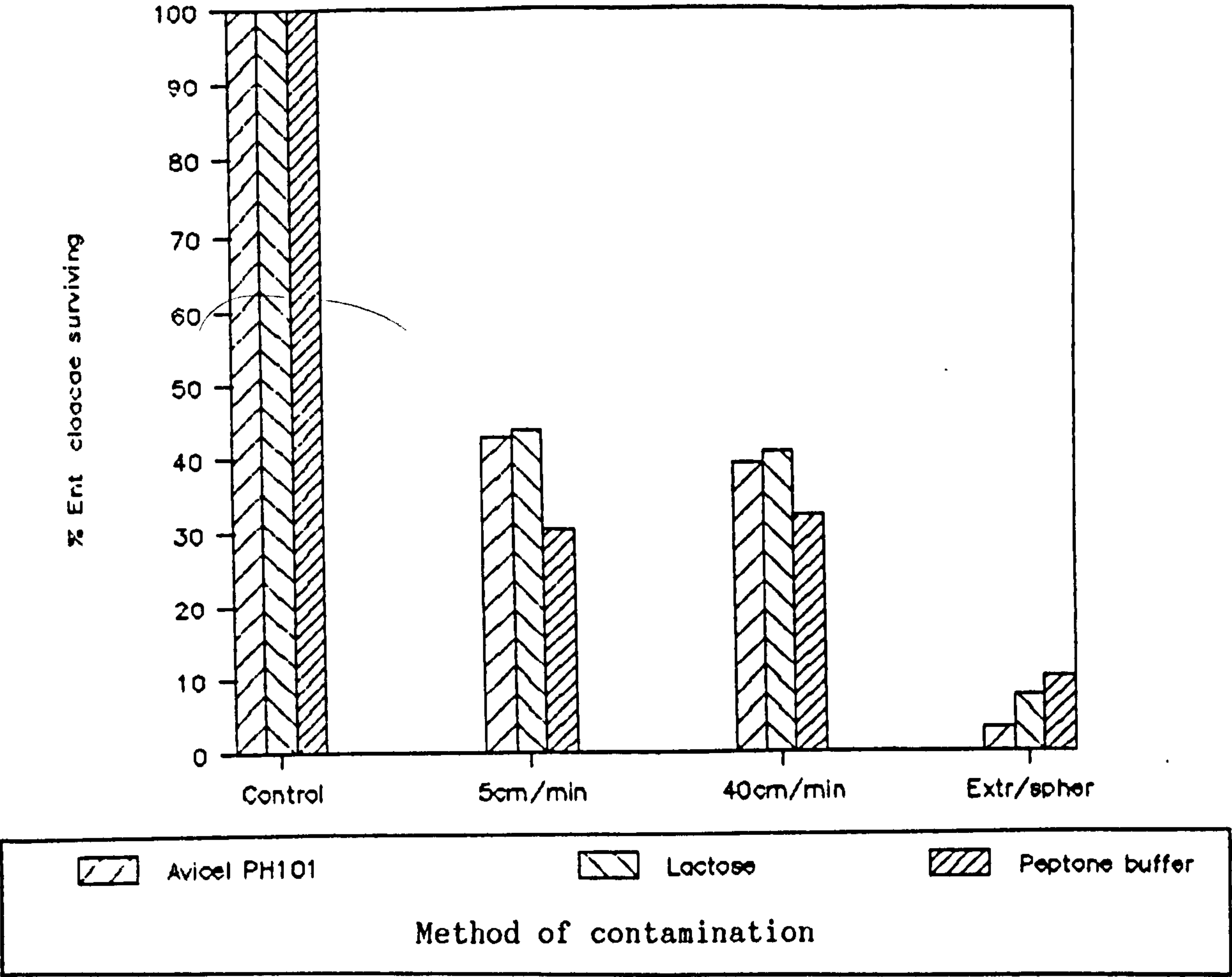
Method of contamination	Viable count of extrudate	Viable count of spheroids	% Survivors in spheroids compared with extrudate
(i) Peptone buffer	$1.80 \times 10^9$	$5.78 \times 10^8$	8.39
(ii) Lactose	$9.36 \times 10^9$	$1.75 \times 10^9$	18.70
(iii) Avicel PH101	$2.80 \times 10^9$	$2.35 \times 10^8$	32.11

Table 5.6 The survival of Ent. cloacae on extrusion and subsequent spheronisation, according to the route of contamination

<u>Survivors as a percentage of contamination in the unextruded material</u>				
<u>Method of contamination</u>	Control	5cm/min extrude	40cm/min extrude	40cm/min extrude + spheronise
(i) Peptone buffer	100	30.47	32.26	10.36
(ii) Lactose	100	43.74	40.90	7.66
(iii) Avicel PH101	100	42.86	39.20	3.29



Figure 5.5 Chart showing the levels of Ent. cloacae surviving extrusion and subsequent spheronisation, according to the method of contamination



Control = % survival in unprocessed material

5cm/min = % survival in material extruded at 5cm/minute

40cm/min = % survival in material extruded at 40cm/minute

Extr/spher = % survival in material extruded at 40cm/minute,  
followed by spheronisation

Ent. cloacae with suspensions of Avicel PH101 in distilled water (section 3.1.2), it is assumed that lactose was the carbon source in this system. Bacterial growth was significantly enhanced where peptone buffer was used as the granulating fluid, presenting a more readily-available source of nutrients.

It has been demonstrated that the rheological properties of the type of extrusion formulation used in this investigation, change in the hours following the addition of water (Raines, unpublished; Fielden, 1987). This is thought to be due to the time-dependent equilibration of added water with components of the formulation, and partial dissolution of lactose (Fielden, 1987). To prepare extrudate where the rheological uniformity of batches is critical, a delay of six to eight hours between mixing and extruding the wet mixture may be necessary. In such cases, the possibility of microbial proliferation in the wet powder mass and processing equipment should be considered.

Wet powder mass, contaminated with a working suspension of Ent. cloacae in peptone buffer as the granulating fluid, was extruded at a constant ram speed of 10cm/minute. Viable counts were carried out to determine the extent of inactivation in the extrudate, compared with material forced along the extruder barrel, but not actually extruded.

The force-displacement profiles for the process show a fairly long compression phase, followed by a short, but quite discreet steady state flow stage, and an extensive forced flow stage (figure 5.3). The early development of forced flow may be a result of the

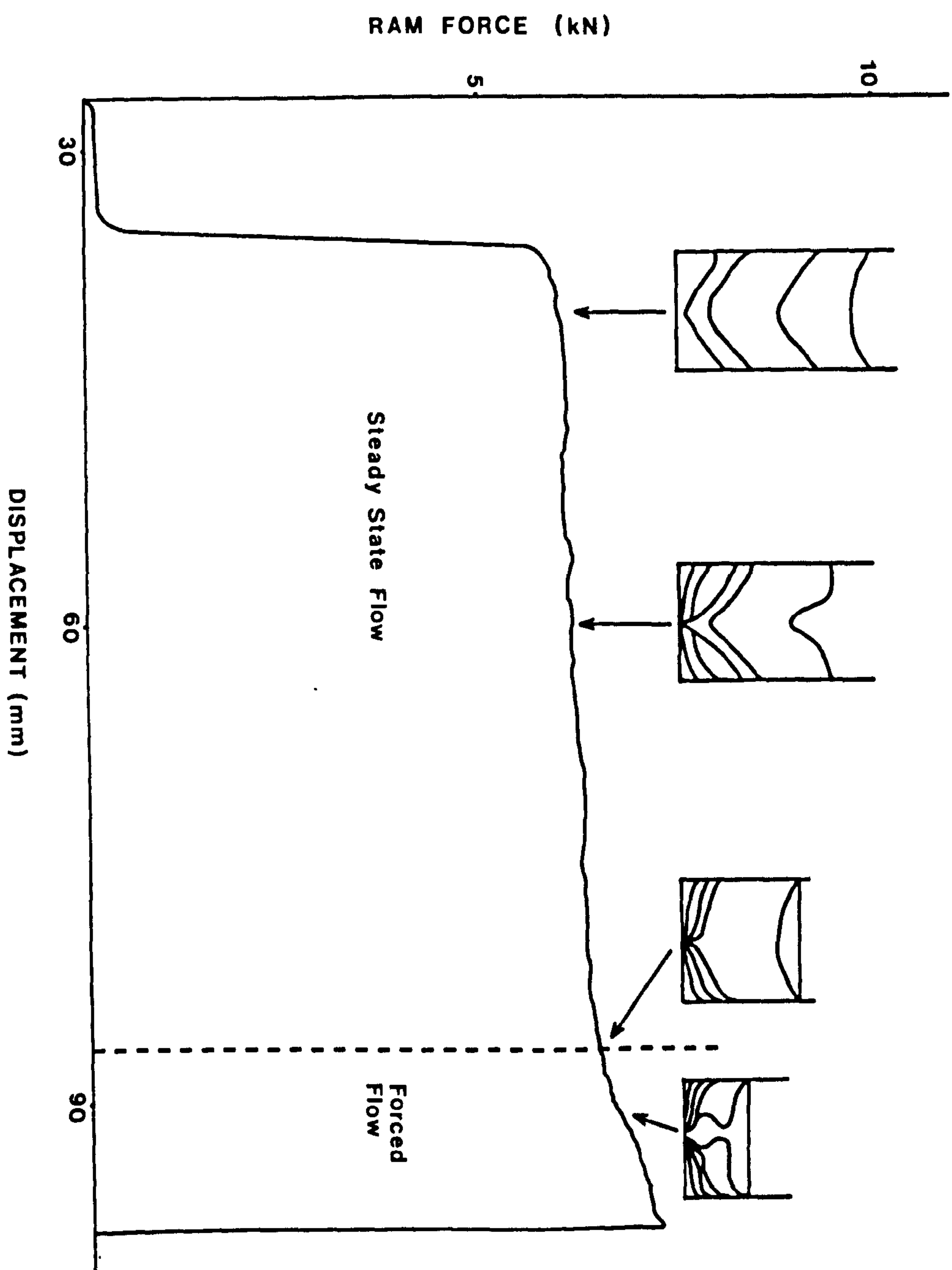
relatively slow ram speed used, since this can allow the build up of a moisture gradient within the wet powder mass (Harrison et al, 1985). The barrel contents were sampled during steady state flow, when a compression force of approximately 2 kN (4 MPa for the piston diameter used) was recorded. This represents the uniform force required to maintain constant flow towards the die.

Results shown on table 5.2 indicate that there was a marked difference between the percentage of organisms inactivated in material within the centre of the extruder barrel, and in material situated at the barrel wall. The greater extent of inactivation in material at the barrel wall can be explained by the unequal distribution of forces acting on the flowing material. It has been demonstrated by Harrison et al (1984) that during steady state flow, the wet powder mass adopts a convergent flow pattern towards the central die hole, as illustrated by figure 5.6. This is quite different from the laminar flow of liquid through a constant tube, and is caused by viscous drag of the wet powder mass at the barrel wall. The result is that during convergent flow, the yield pressure (required to initiate flow) is lower for material moving along the established flow patterns towards the die, and relatively higher for material near the barrel wall where the viscous retarding force is greatest. Thus material at the barrel wall experiences a greater compression force before it begins to flow. This contributes to a higher shear stress, the extent of which is proportional to the distance from the centre of the barrel tube (equation 24), and overall leads to the increased



Figure 5.6 Force-displacement profile showing convergent flow pattern into the die

(after Harrison, 1982).



bacterial kill in material at the barrel wall.

The inactivation of bacteria in the steady state extrudate was appreciably higher than for unextruded material (table 5.2). On extrusion, the wet mass is forced to deform on entry to the die, and during flow through the die capillary it is subjected to shear stresses which are again greatest at the die wall. According to the results of Fielden (1987), the die wall shear stress for this formulation, extruded at 10cm/minute, would be about 0.3 MPa for the piston diameter used. Plates 4 and 5 illustrate the deformation of the extruded material at the die wall, compared with material within the cross-section of the extrudate.

On exiting the die, material extruded against the die wall may undergo a degree of elastic recovery which results in a finite amount of further, localized shear flow. The loss in viability of bacteria in the extruded material was compared with the degree of inactivation during forced flow, when compression forces of up to 12 kN were recorded (equivalent to about 24 MPa for the piston diameter used). It was found that a slightly higher proportion of micro-organisms were inactivated during extrusion, where shear is the dominant stress, than during forced flow within the barrel, where chronic, increasing compression forces predominate.

A series of experiments were carried out to compare bacterial inactivation during ram extrusion and spheronisation of wet powder masses, which had been contaminated with Ent. cloacae by different methods. Harrison (1982) reported that extrusion force was dependent upon the extrusion rate. In these studies, the

relationship between extrusion rate and extrusion force was investigated, by comparing the forces recorded for extrusion at 5cm/minute and 40cm/minute. The results are shown on table 5.4, and demonstrate that despite a higher extrusion force at 40cm/minute, bacterial kill was no greater than at the lower speed (5cm/minute). The reason may be that the wall shear stress at 40cm/minute is not very much greater than at 5cm/minute, as shown on table 5.7.

There was no significant difference in the degree of microbial inactivation observed for extrudate prepared by using contaminated lactose or Avicel PH101, but for both ram speeds much greater inactivation was noted where contaminated peptone buffer was used as the granulating fluid. This may be because previous growth in suspensions of Avicel PH101 or lactose encourages attachment of the micro-organisms onto or within the powder particles, which affords some degree of protection from shear.

During spheronisation, frictional forces from the rotating plate, and the plasticity of Avicel PH101, shape and densify the extrudate into spheres. Maximum inactivation of the contaminating organisms was observed for spheroids where the original wet powder mass was prepared from contaminated Avicel PH101. The possible reason for this is that some of the micro-organisms grown with Avicel PH101 have escaped from the effects of previous compression by residing within void spaces in the structure of the microcrystalline cellulose particles. These micro-organisms may then be inactivated during the densification of Avicel PH101 which



Table 5.7    The effect of varying ram speed on the die wall shear stress,  $\tau_w$ , and the upstream pressure loss,  $p_o$ .

(Fielden, 1987).

Ram speed (cm/min)	$\tau_w$ (kNm <sup>-2</sup> )	$p_o$ (kNm <sup>-2</sup> )
40	325	6096
20	310	5167
10	300	4568
* 5	* 295	* 4200

\* extrapolated values

accompanies spheronisation and allows the formation of spheroids.

In previous discussions of tablet compression, shear has been cited as a mediator of kill (Plumpton et al, 1986b; Fassihi and Parker, 1987), together with thermal effects, frictional forces and fracture propagation (Fassihi and Parker, 1987). Ram extrusion of a wet powder mass, is considered to be an isothermal process (Harrison, 1982), therefore thermal effects are unlikely to contribute towards microbial kill. In these investigations, the compression forces achieved did not exceed 24 MPa, so failing to reach the fracture threshold for Avicel PH101. The aqueous phase would be expected to have a lubricating effect and partially dissolve the lactose, thereby reducing the magnitude of frictional forces between the powder particles. It is considered that the results of these experiments indicate that shearing forces make a significant contribution towards microbial kill during the processing of contaminated excipient powders into solid dosage forms.

6. THE SURVIVAL OF BACTERIA IN TABLETS STORED AT A  
RANGE OF RELATIVE HUMIDITIES



## 6.1 METHODS AND RESULTS

### 6.1.1 Introduction

Tablets contaminated with viable micro-organisms may be considered to be adequately preserved so long as they are kept dry. Spore-forming bacteria and fungi are capable of protecting themselves from conditions of drought, and can survive for long periods of time in powders and tablets. The behaviour of strictly-vegetative bacteria in dry solid oral dosage forms has not been extensively studied, despite the fact that the bacteria specified in the British Pharmacopoeia (1988) "Tests for Microbial Contamination" are not spore-forming. The aim of the work that follows is to investigate the effect of water availability on the survival of vegetative bacteria in "dry" tablet systems.

The organisms selected, Ent. cloacae and Staph. aureus, are non-spore forming, and have different minimum water requirements for growth. The tablet excipients were selected to allow systems with different hygroscopicities to be studied, and were contaminated with a working suspensions of peptone buffer, to provide a nutrient source for the bacteria.

### 6.1.2 Constant humidity environments for storage

The contaminated tablets were placed in constant humidity chambers, incubated at 25°C. The chambers were pyrex desiccators sealed with silicone vacuum grease, and the chamber relative humidity was controlled by placing an open dish of a suitable saturated salt solution (with excess crystals), or sulphuric acid

solution, at the bottom of the chamber. The tablet samples, in open glass jars, rested on a metal grid above the humidity-controlling solution.

#### 6.1.2.1 Saturated salt solutions

Saturated salt solutions were prepared according to published tables of humidity-controlling solutions (Stokes and Robinson, 1949; Handbook of Chemistry and Physics, 44th Edn., The Chemical Rubber Co.). Triple distilled water (from an all-glass still) was used, and all chemicals were of analytical grade (BDH Ltd.). The salts were selected to produce solutions with relative humidities as listed on table 6.1, at 25°C.

#### 6.1.2.2 Sulphuric acid solutions

Solutions of sulphuric acid in triple distilled water were prepared according to published tables (Stokes and Robinson, 1949), and used to produce a range of high relative humidity atmospheres as shown on table 6.2.

#### 6.1.2.3 Rotronic Hygroskop

The relative humidities of the controlling solutions were checked periodically using the Rotronic Hygroskop operating at 25°C, allowing 60 minutes for equilibration of the relative humidity in the Hygroskop chamber.

The Hygroskop itself was calibrated three-monthly using a set of lithium chloride standards supplied by the manufacturer. For most

Table 6.1 Relative humidities of saturated salt solutions  
at 25°C

Solid phase		Measured % Relative Humidity
Potassium acetate	$K(C_2H_3O_2) \cdot 1.5H_2O$	23.5
Potassium carbonate	$K_2CO_3 \cdot 2H_2O$	45.8
Sodium nitrite	$NaNO_2$	66.5
Ammonium chloride	$NH_4Cl$	78.6
Potassium chloride	$KCl$	86.4
Sodium sulphate	$Na_2SO_4 \cdot 10H_2O$	93.3

Table 6.2 Relative humidities of Sulphuric acid solutions  
at 25°C

$H_2SO_4$ (anhydrous)		% Relative humidity
Moles /1000g water	% w/v	
3.73	26.8	80
3.03	22.9	85
2.22	17.9	90
1.26	11.0	95



purposes, the device was calibrated to maximum accuracy (+/- 0.5%RH) using a 50%RH LiCl solution, which gave a measured error of 1.9% at 100%RH (triple distilled water). However, for studies involving tablets stored at high relative humidities, the Hygroskop was calibrated to 80 or 95%RH.

### 6.1.3 Production and storage of contaminated tablets

Tablets were produced from contaminated excipients at suitable, low compression settings, as described in section 4.1, and stored in constant humidity chambers at 25°C for two to three weeks.

Viable counts of tablet samples were determined as described previously (section 2.3), immediately after tableting, and at intervals during storage, using two samples of three weighed tablets (about 1g). At the same times, a batch of five tablets was withdrawn from each humidity chamber, and the RH measured to ensure that the tablets were in equilibrium with the  $A_w$  of the controlling solution. The tablets were found to require 30 minutes in the Hygroskop chamber for equilibration of the RH reading.

#### 6.1.3.1 The behaviour of *Ent. cloacae* and *Staph. aureus* in tablets prepared from three different excipients and stored at a range of $A_w$ conditions

##### Method

Tablets contaminated with *Ent. cloacae* or *Staph. aureus* were produced from maize starch, Avicel PH101 or lactose, containing 1% magnesium stearate as lubricant. Storage and viable counting procedures were as described in section 6.1.3.

## Results

The patterns of survival are shown in figures 6.1 to 6.12. The tableting compressions and viable count results are shown in tables 6.3 to 6.14.

Ent. cloacae was able to grow in lactose, Avicel PH101 and maize starch Avicel tablets in RH conditions of 100%. Staph. aureus did not show marked growth in any of the tablets.

Growth was not observed at Aw levels less than 1.00 in any of the systems studied. Bacterial survival was better at lower water activities, between 0.23 and 0.66Aw. Bacterial death rates were highest at Aw levels which approached 1.00. The bacteria survived best across a range of Aw in maize starch tablets. For this reason, the study was extended to investigate the behaviour of Ent. cloacae in other starches.

### 6.1.3.2 The behaviour of Ent. cloacae in tablets prepared from wheat starch and potato starch, and stored at a range of Aw conditions

## Method

Tablets contaminated with Ent. cloacae were prepared from wheat starch or potato starch, with 1% magnesium stearate as lubricant. Storage and viable counting conditions were as described above (6.1.3). Survival was compared with that shown by Ent. cloacae in maize starch tablets (figures 6.1 and 6.3, tables 6.3 and 6.5).

## Results

Figures 6.13 to 6.16 show the patterns of Ent. cloacae survival,

and the viable count results are shown on tables 6.15 to 6.18.

By comparison with results previously obtained for Ent. cloacae in maize starch tablets (section 6.1.3.1), it was apparent that bacterial survival was similar in maize starch, wheat starch and potato starch tablets. In each case, growth of Ent. cloacae was observed in conditions where the  $A_w$  was 1.00, but the organisms gradually died at 0.93 $A_w$ . The bacteria survived well at a range of  $A_w$ , from 0.23 to 0.79, in each of the three starches, and up to 0.86 $A_w$  in potato and wheat starch.

## 6.2 DISCUSSION OF RESULTS

The patterns of bacterial survival in stored contaminated tablets are shown in figures 6.1 to 6.16, and vary according to the excipient used and the type of bacteria present.

When stored at a range of relative humidities, the microbial contaminants in tablets prepared from maize starch, Avicel PH101 and lactose behaved quite differently (figures 6.1 to 6.12). For the tablets stored over distilled water, with a potential  $A_w$  of 1.00, Ent. cloacae was able to grow in Avicel PH101, lactose and maize starch. For lactose tablets at 1.00 $A_w$ , condensed moisture produced a film of concentrated lactose solution on the tablet surface, and growth of Ent. cloacae was followed by overgrowth by moulds which originated from the air of the humidity chamber.

Staph. aureus grew to some extent in maize starch tablets, but showed little growth at 1.00  $A_w$  in the other tablet systems. Failure to grow in an environment where free water is available



Figure 6.1 Patterns of survival for Ent. cloacae contaminating maize starch tablets in a range of relative humidities, at 25°C

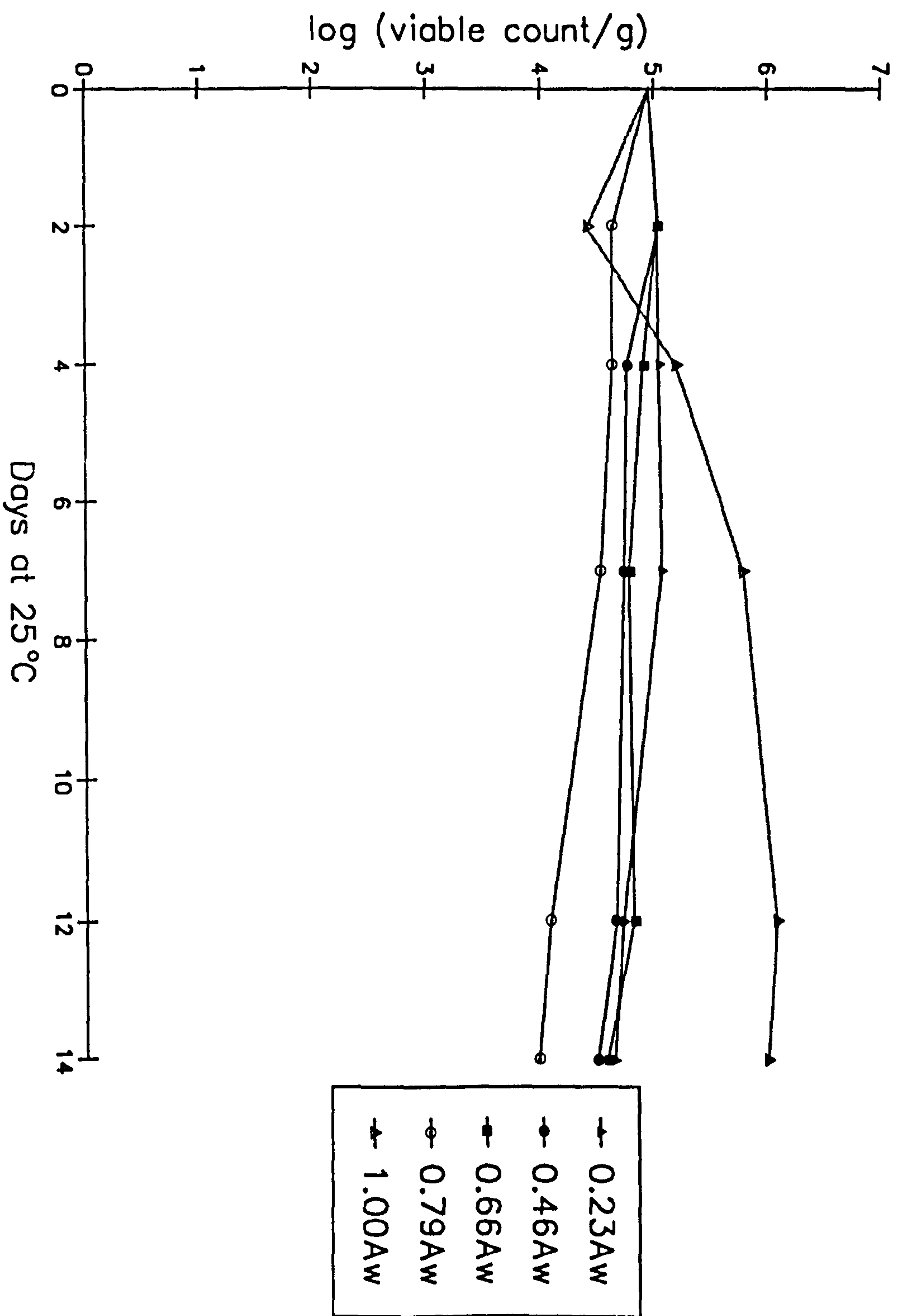


Table 6.3 Viable counts (cfu/g) and [log viable counts] for  
Maize starch tablets with Ent. cloacae

Days at 25°C	Aw of storage				
	0.23	0.46	0.66	0.79	1.00
0	9.11x10 <sup>4</sup> [4.96]	9.11x10 <sup>4</sup> [4.96]	9.11x10 <sup>4</sup> [4.96]	9.11x10 <sup>4</sup> [4.96]	9.11x10 <sup>4</sup> [4.96]
2	1.13x10 <sup>5</sup> [5.05]	1.09x10 <sup>5</sup> [5.04]	1.09x10 <sup>5</sup> [5.04]	4.36x10 <sup>4</sup> [4.64]	2.62x10 <sup>4</sup> [4.42]
4	5.93x10 <sup>4</sup> [4.77]	1.12x10 <sup>5</sup> [5.05]	8.23x10 <sup>4</sup> [4.91]	4.36x10 <sup>4</sup> [4.64]	1.58x10 <sup>5</sup> [5.20]
7	5.48x10 <sup>4</sup> [4.74]	1.20x10 <sup>5</sup> [5.07]	6.60x10 <sup>4</sup> [4.78]	3.40x10 <sup>4</sup> [4.53]	6.03x10 <sup>5</sup> [5.78]
12	4.50x10 <sup>4</sup> [4.66]	5.29x10 <sup>4</sup> [4.72]	6.42x10 <sup>4</sup> [4.81]	1.21x10 <sup>4</sup> [4.08]	1.15x10 <sup>6</sup> [6.06]
14	3.13x10 <sup>4</sup> [4.49]	4.41x10 <sup>4</sup> [4.64]	3.73x10 <sup>4</sup> [4.57]	9.42x10 <sup>3</sup> [3.97]	9.33x10 <sup>5</sup> [5.97]

Tablets compressed at 4.50 kN (52 MPa).

Figure 6.2 Patterns of survival for Staph. aureus contaminating maize starch tablets in a range of relative humidities, at 25°C

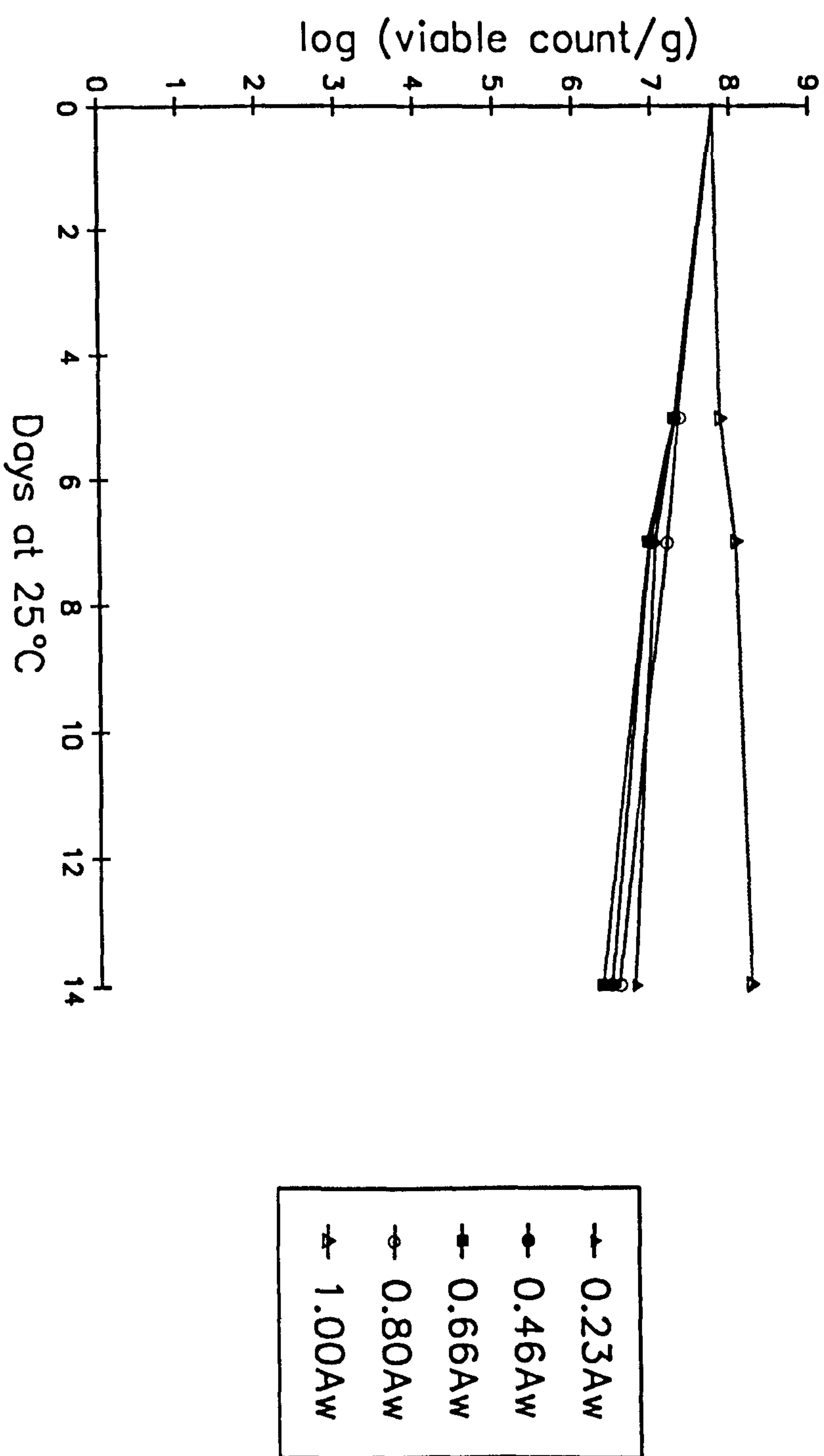




Table 6.4 Viable counts (log cfu/g) and [log viable counts] for  
Maize starch tablets with Staph. aureus

Days at 25°C	Aw of storage				
	0.23	0.46	0.66	0.80	1.00
0	6.17x10 <sup>7</sup> [7.79]	6.17x10 <sup>7</sup> [7.79]	6.17x10 <sup>7</sup> [7.79]	6.17x10 <sup>7</sup> [7.79]	6.17x10 <sup>7</sup> [7.79]
5	1.97x10 <sup>7</sup> [7.29]	1.96x10 <sup>7</sup> [7.29]	2.03x10 <sup>7</sup> [7.31]	2.18x10 <sup>7</sup> [7.34]	7.51x10 <sup>7</sup> [7.87]
7	1.13x10 <sup>7</sup> [7.05]	9.85x10 <sup>6</sup> [6.99]	1.13x10 <sup>7</sup> [7.05]	1.58x10 <sup>7</sup> [7.20]	1.17x10 <sup>8</sup> [8.07]
14	5.59x10 <sup>6</sup> [6.75]	2.89x10 <sup>6</sup> [6.46]	2.48x10 <sup>6</sup> [6.39]	3.59x10 <sup>6</sup> [6.55]	1.70x10 <sup>8</sup> [8.23]

Tablets compressed at 3.32 kN (38 MPa).

Figure 6.3 Patterns of survival for Ent. cloacae contaminating maize starch tablets in a range of high relative humidities, at 25°C

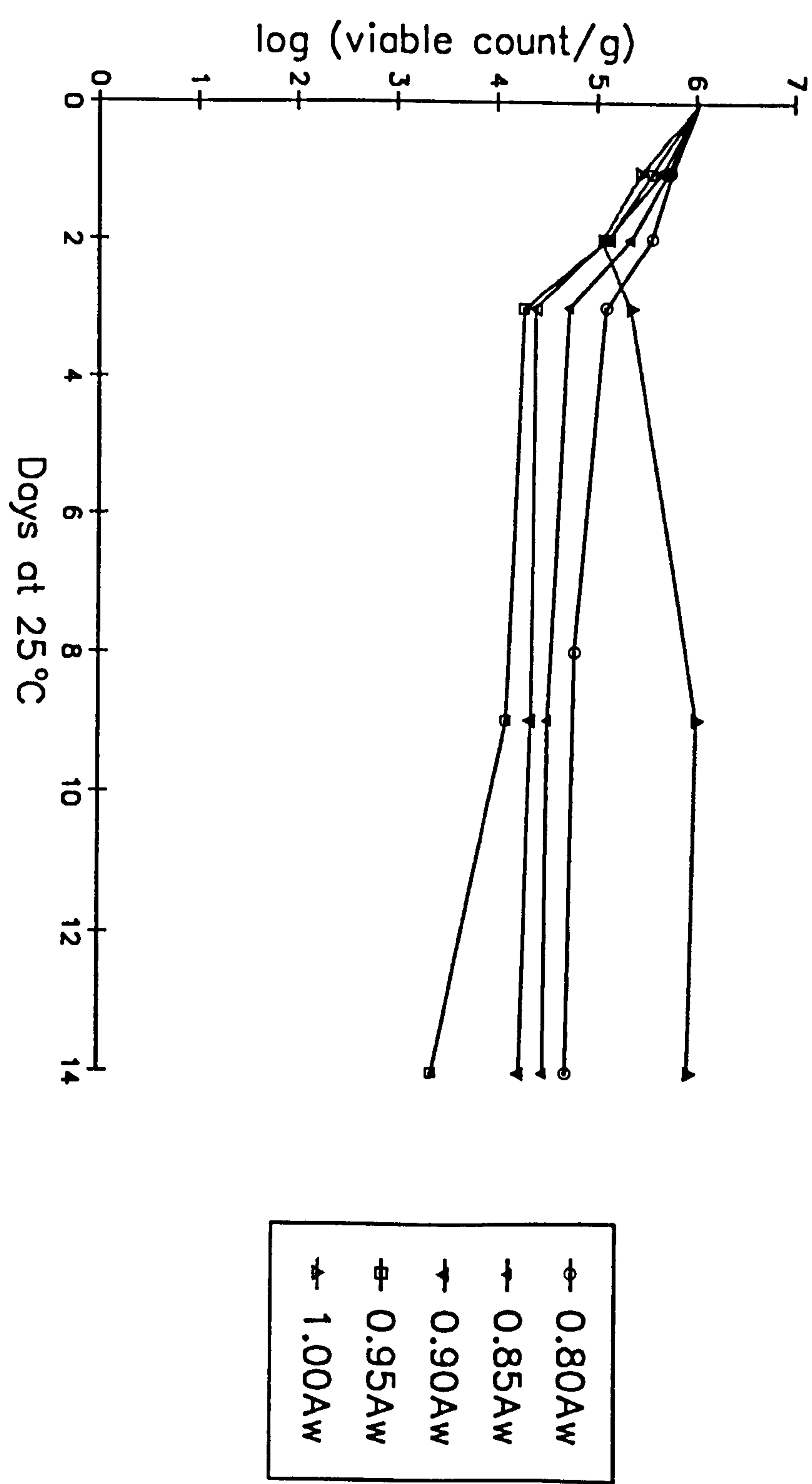


Table 6.5 Viable counts and [log viable counts] for  
Maize starch tablets with Ent. cloacae at high Aw.

Days at 25°C	Aw of storage			
	0.79	0.86	0.93	1.00
0	9.11x10 <sup>4</sup> [4.96]	9.11x10 <sup>4</sup> [4.96]	9.11x10 <sup>4</sup> [4.96]	9.11x10 <sup>4</sup> [4.96]
2	4.36x10 <sup>4</sup> [4.64]	3.73x10 <sup>4</sup> [4.57]	5.36x10 <sup>4</sup> [4.72]	2.62x10 <sup>4</sup> [4.42]
4	4.36x10 <sup>4</sup> [4.64]	1.89x10 <sup>4</sup> [4.27]	5.90x10 <sup>3</sup> [3.77]	1.58x10 <sup>5</sup> [5.20]
7	3.40x10 <sup>4</sup> [4.53]	1.02x10 <sup>4</sup> [4.01]	1.17x10 <sup>3</sup> [3.07]	6.03x10 <sup>5</sup> [5.78]
12	1.21x10 <sup>4</sup> [4.08]	1.83x10 <sup>3</sup> [3.26]	1.17x10 <sup>2</sup> [2.07]	1.15x10 <sup>6</sup> [5.97]

Tablets compressed at 4.23 kN (38.3 MPa).



Figure 6.4 Patterns of survival for Staph. aureus contaminating maize starch tablets in a range of high relative humidities, at 25°C

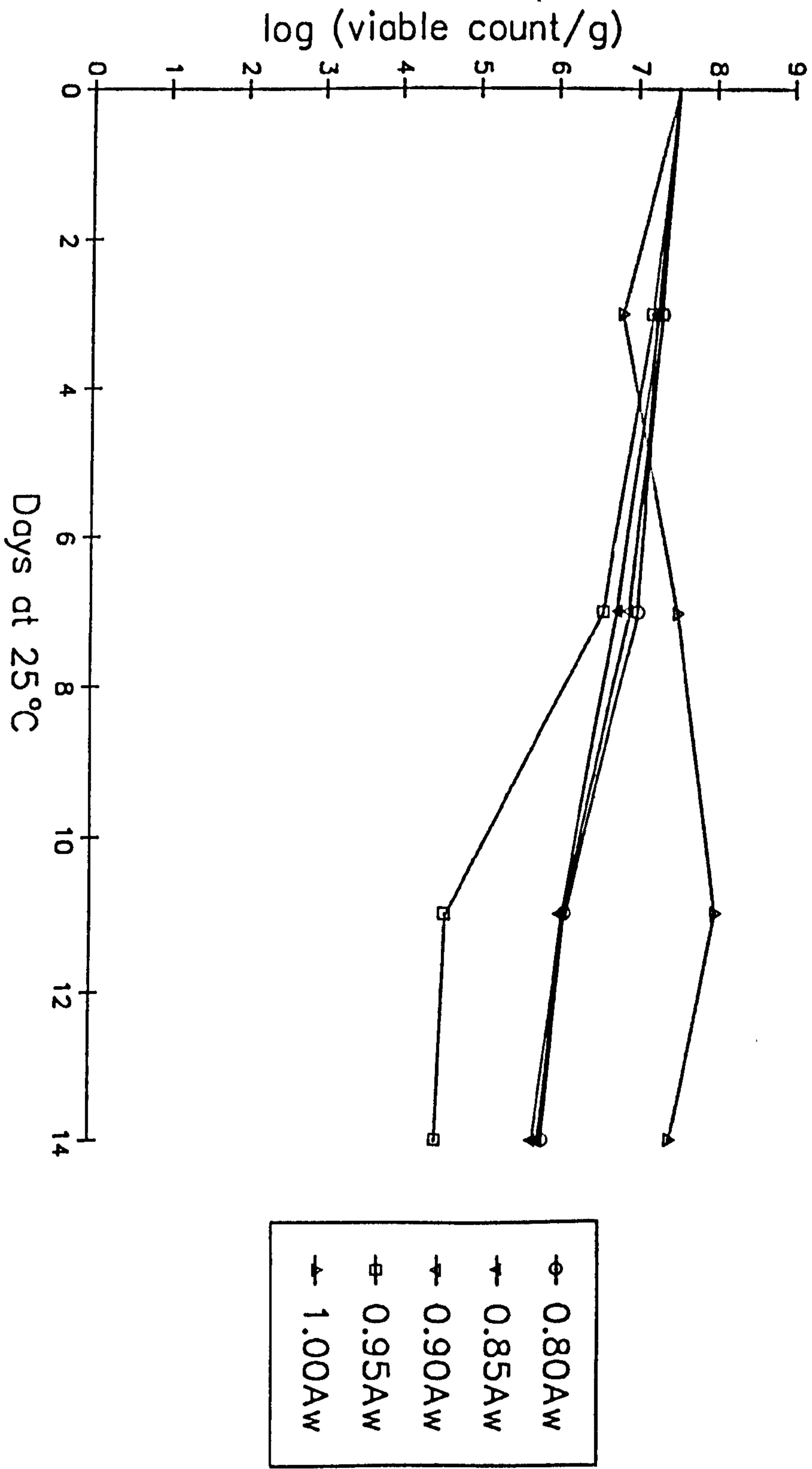


Table 6.6 Viable counts (cfu/g) and [log viable counts] for  
Maize starch tablets with Staph. aureus at high Aw.

Days at 25°C	Aw of storage				
	0.80	0.85	0.90	0.95	1.00
0	3.30x10 <sup>7</sup> [7.52]	3.30x10 <sup>7</sup> [7.52]	3.30x10 <sup>7</sup> [7.52]	3.3x10 <sup>7</sup> [7.52]	3.30x10 <sup>7</sup> [7.52]
3	2.12x10 <sup>7</sup> [7.33]	1.95x10 <sup>7</sup> [7.29]	1.85x10 <sup>7</sup> [7.27]	1.63x10 <sup>7</sup> [7.21]	6.77x10 <sup>6</sup> [6.83]
7	1.04x10 <sup>7</sup> [7.02]	5.98x10 <sup>6</sup> [6.78]	1.02x10 <sup>7</sup> [7.01]	3.98x10 <sup>6</sup> [6.60]	3.64x10 <sup>7</sup> [7.56]
11	1.33x10 <sup>6</sup> [6.12]	1.23x10 <sup>6</sup> [6.09]	1.30x10 <sup>6</sup> [6.11]	3.88x10 <sup>4</sup> [4.59]	1.14x10 <sup>8</sup> [8.06]
14	6.73x10 <sup>5</sup> [5.83]	5.22x10 <sup>5</sup> [5.72]	7.11x10 <sup>5</sup> [5.85]	2.96x10 <sup>4</sup> [4.47]	3.16x10 <sup>7</sup> [7.50]

Tablets compressed at 3.32 kN (38.3 MPa).

Figure 6.5 Patterns of survival for Ent. cloacae contaminating Avicel PH101 tablets in a range of relative humidities, at 25°C

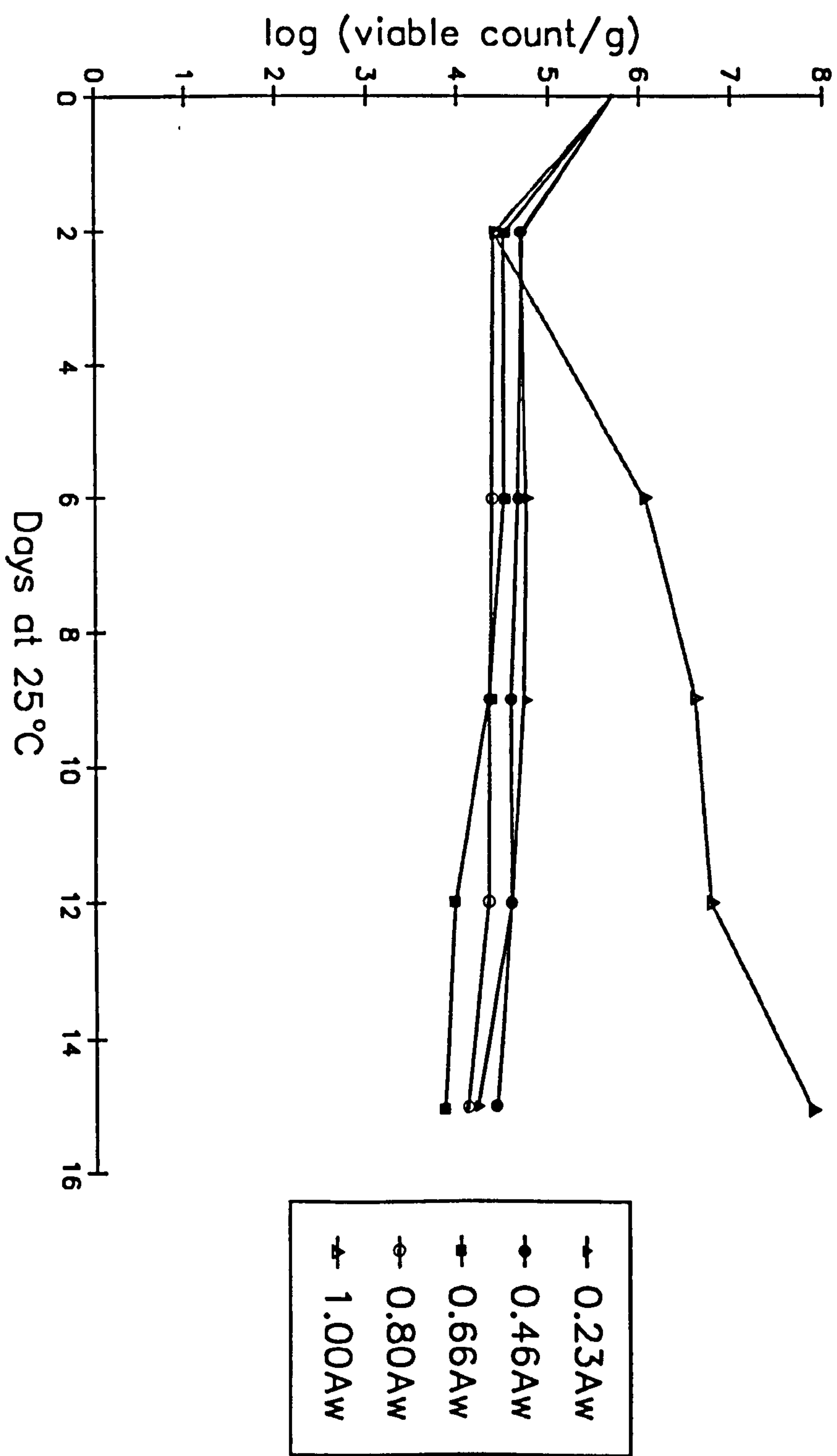




Table 6.7 Viable counts (cfu/g) and [log viable counts] for  
Avicel PH101 tablets with Ent. cloacae

Days at 25°C	Aw of storage				
	0.23	0.46	0.66	0.80	1.00
0	5.10x10 <sup>5</sup> [5.71]	5.10x10 <sup>5</sup> [5.71]	5.10x10 <sup>5</sup> [5.71]	5.10x10 <sup>5</sup> [5.71]	5.10x10 <sup>5</sup> [5.71]
2	4.87x10 <sup>4</sup> [4.69]	5.15x10 <sup>4</sup> [4.71]	3.26x10 <sup>4</sup> [4.51]	2.49x10 <sup>4</sup> [4.40]	2.50x10 <sup>4</sup> [4.40]
6	5.71x10 <sup>4</sup> [4.75]	4.44x10 <sup>4</sup> [4.65]	3.19x10 <sup>4</sup> [4.50]	2.33x10 <sup>4</sup> [4.37]	1.15x10 <sup>6</sup> [6.06]
9	5.32x10 <sup>4</sup> [4.72]	3.72x10 <sup>4</sup> [4.57]	2.12x10 <sup>4</sup> [4.33]	2.17x10 <sup>4</sup> [4.34]	4.03x10 <sup>6</sup> [6.61]
12	3.92x10 <sup>4</sup> [4.59]	3.79x10 <sup>4</sup> [4.58]	9.19x10 <sup>3</sup> [3.96]	2.15x10 <sup>4</sup> [4.33]	6.09x10 <sup>6</sup> [6.78]
15	1.59x10 <sup>4</sup> [4.20]	2.55x10 <sup>4</sup> [4.41]	7.00x10 <sup>3</sup> [3.84]	1.24x10 <sup>4</sup> [4.09]	7.42x10 <sup>7</sup> [7.87]

Tablets compressed at 0.25 kN (2.90 MPa).

Figure 6.6 Patterns of survival for Staph. aureus contaminating Avicel PH101 tablets in a range of relative humidities, at 25°C

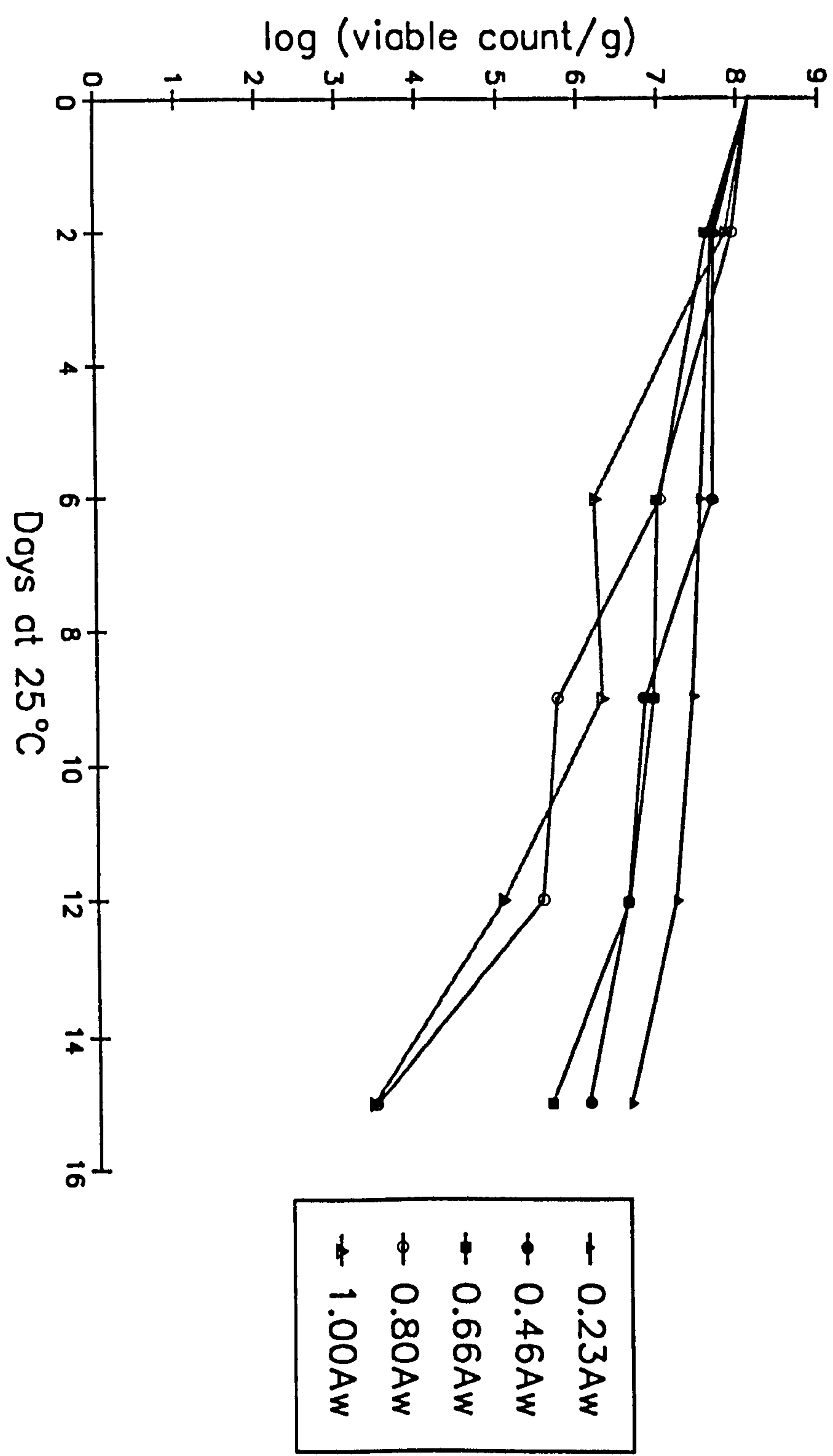


Table 6.8 Viable counts (cfu/g) and [log viable counts] for Avicel PH101 tablets with Staph. aureus.

Days at 25°C	Aw of storage				
	0.23	0.46	0.66	0.80	1.00
0	1.42x10 <sup>8</sup> [8.15]	1.42x10 <sup>8</sup> [8.15]	1.42x10 <sup>8</sup> [8.15]	1.42x10 <sup>8</sup> [8.15]	1.42x10 <sup>8</sup> [8.15]
2	5.78x10 <sup>7</sup> [7.76]	5.12x10 <sup>7</sup> [7.71]	8.55x10 <sup>7</sup> [7.93]	4.12x10 <sup>7</sup> [7.62]	7.12x10 <sup>7</sup> [7.85]
6	3.42x10 <sup>7</sup> [7.53]	4.85x10 <sup>7</sup> [7.68]	9.78x10 <sup>6</sup> [6.99]	1.08x10 <sup>7</sup> [7.03]	1.60x10 <sup>6</sup> [6.20]
9	2.62x10 <sup>7</sup> [7.42]	6.84x10 <sup>6</sup> [6.83]	8.54x10 <sup>6</sup> [6.93]	5.35x10 <sup>5</sup> [5.73]	1.94x10 <sup>6</sup> [6.29]
12	1.60x10 <sup>7</sup> [7.20]	3.89x10 <sup>6</sup> [6.59]	4.12x10 <sup>6</sup> [6.61]	3.28x10 <sup>5</sup> [5.52]	1.07x10 <sup>5</sup> [5.03]
15	4.06x10 <sup>6</sup> [6.61]	1.21x10 <sup>6</sup> [6.08]	4.07x10 <sup>5</sup> [5.61]	2.63x10 <sup>3</sup> [3.42]	2.43x10 <sup>3</sup> [3.38]

Tablets compressed at 0.54 kN (6.20 MPa).



Figure 6.7 Patterns of survival for Ent. cloacae contaminating Avicel PH101 tablets in a range of high relative humidities, at 25°C

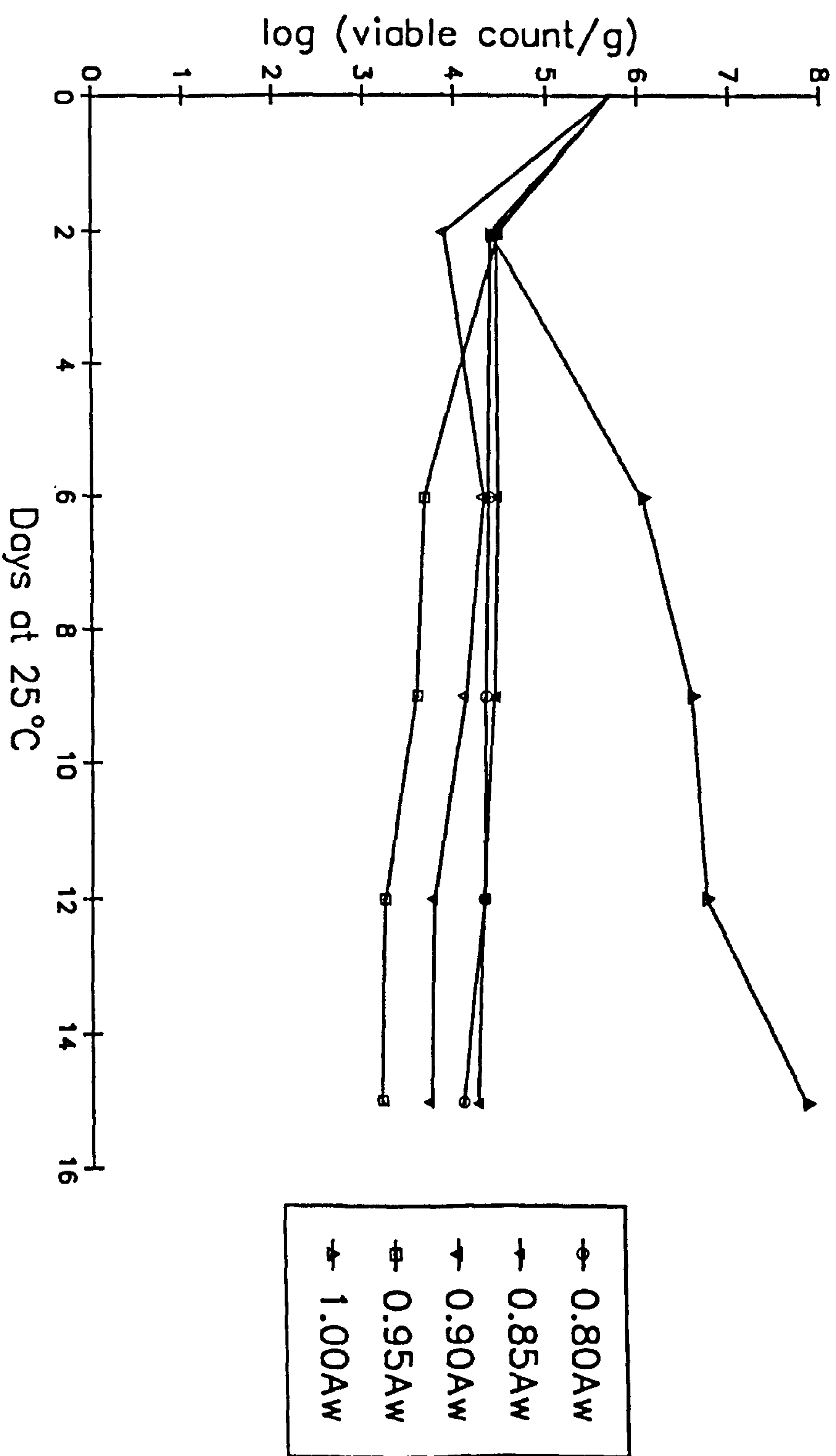


Table 6.9 Viable counts (cfu/g) and [log viable counts] for Avicel PH101 tablets with Ent. cloacae at high Aw

Days at 25°C	Aw of storage				
	0.80	0.85	0.90	0.95	1.00
0	5.10x10 <sup>5</sup> [5.71]	5.10x10 <sup>5</sup> [5.71]	5.10x10 <sup>5</sup> [5.71]	5.10x10 <sup>5</sup> [5.71]	5.10x10 <sup>5</sup> [5.71]
2	2.49x10 <sup>4</sup> [4.40]	2.88x10 <sup>4</sup> [4.46]	8.02x10 <sup>3</sup> [3.90]	3.14x10 <sup>4</sup> [4.49]	2.50x10 <sup>4</sup> [4.40]
6	2.33x10 <sup>4</sup> [4.37]	2.95x10 <sup>4</sup> [4.47]	2.07x10 <sup>4</sup> [4.32]	4.55x10 <sup>3</sup> [3.66]	1.15x10 <sup>6</sup> [6.06]
9	2.17x10 <sup>4</sup> [4.34]	2.69x10 <sup>4</sup> [4.43]	1.32x10 <sup>4</sup> [4.12]	3.72x10 <sup>3</sup> [3.57]	4.03x10 <sup>6</sup> [6.61]
12	2.15x10 <sup>4</sup> [4.33]	2.07x10 <sup>4</sup> [4.32]	5.99x10 <sup>3</sup> [3.77]	1.65x10 <sup>3</sup> [3.22]	6.09x10 <sup>6</sup> [6.78]
15	1.24x10 <sup>4</sup> [4.09]	1.80x10 <sup>4</sup> [4.25]	5.39x10 <sup>3</sup> [3.73]	1.52x10 <sup>3</sup> [3.18]	7.42x10 <sup>7</sup> [7.87]

Tablets compressed at 0.25 kN (2.90 MPa).

Figure 6.8 Patterns of survival for Staph. aureus contaminating Avicel PH101 tablets in a range of high relative humidities, at 25°C

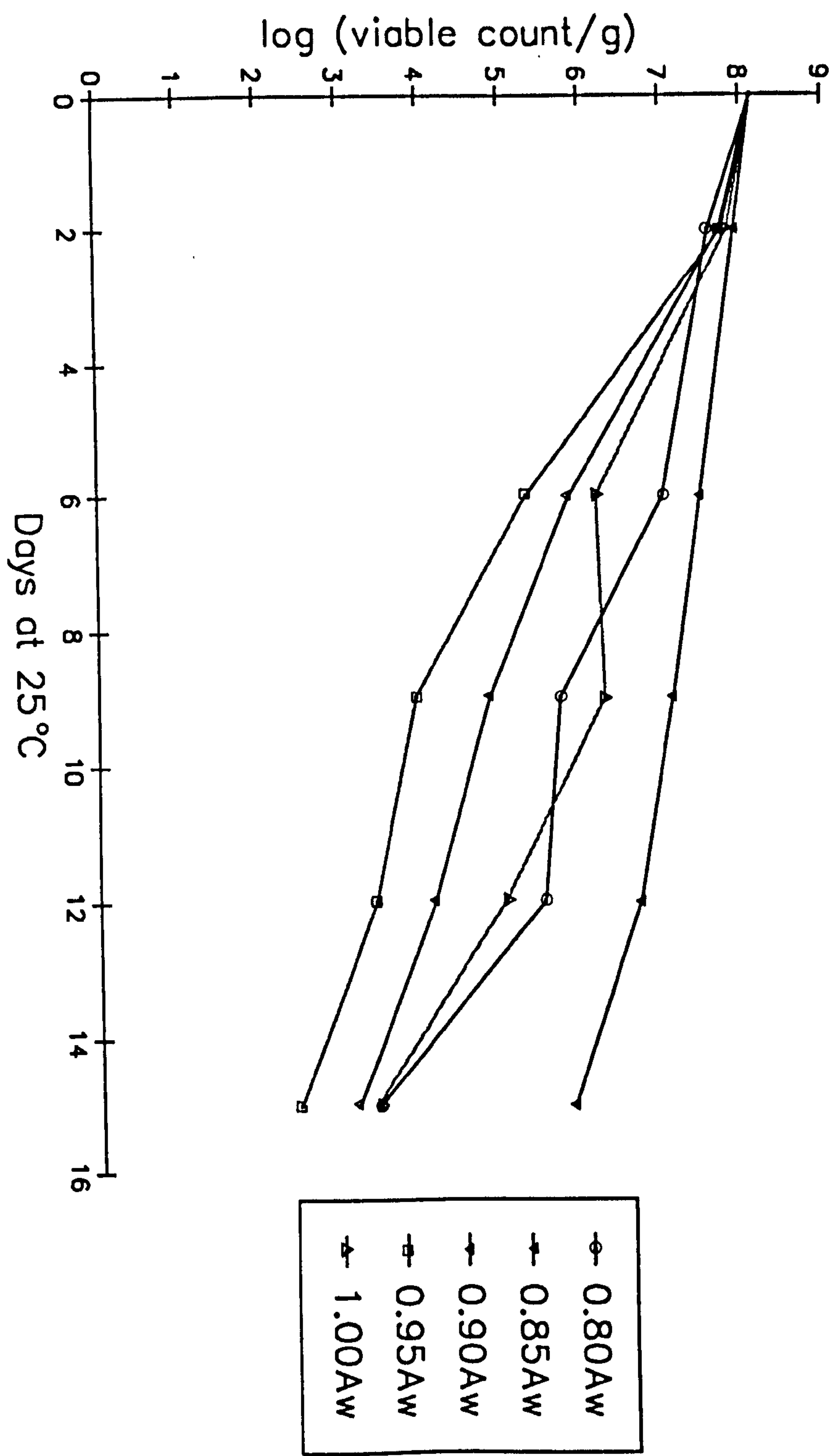




Table 6.10 Viable counts (cfu/g) and [log viable counts] for Avicel PH101 tablets with Staph. aureus at high Aw.

Days at 25°C	Aw of storage				
	0.80	0.85	0.90	0.95	1.00
0	1.42x10 <sup>8</sup> [8.15]	1.42x10 <sup>8</sup> [8.15]	1.42x10 <sup>8</sup> [8.15]	1.42x10 <sup>8</sup> [8.15]	1.42x10 <sup>8</sup> [8.15]
2	4.12x10 <sup>7</sup> [7.62]	8.60x10 <sup>7</sup> [7.93]	5.64x10 <sup>7</sup> [7.75]	6.10x10 <sup>7</sup> [7.78]	7.12x10 <sup>7</sup> [7.85]
6	1.08x10 <sup>7</sup> [7.03]	3.12x10 <sup>7</sup> [7.49]	7.21x10 <sup>5</sup> [5.86]	2.10x10 <sup>5</sup> [5.32]	1.60x10 <sup>6</sup> [6.20]
9	5.35x10 <sup>5</sup> [5.73]	1.38x10 <sup>7</sup> [7.14]	7.02x10 <sup>4</sup> [4.85]	8.54x10 <sup>3</sup> [3.93]	1.94x10 <sup>6</sup> [6.29]
12	3.28x10 <sup>5</sup> [5.52]	5.17x10 <sup>6</sup> [6.71]	1.40x10 <sup>4</sup> [4.15]	2.60x10 <sup>3</sup> [3.41]	1.07x10 <sup>5</sup> [5.03]
15	2.63x10 <sup>3</sup> [3.42]	7.22x10 <sup>5</sup> [5.86]	1.45x10 <sup>3</sup> [3.16]	2.76x10 <sup>2</sup> [2.44]	2.34x10 <sup>3</sup> [3.38]

Tablets compressed at 0.54 kN (6.20 MPa).

Figure 6.9 Patterns of survival for Ent. cloacae contaminating lactose tablets in a range of relative humidities, at 25°C

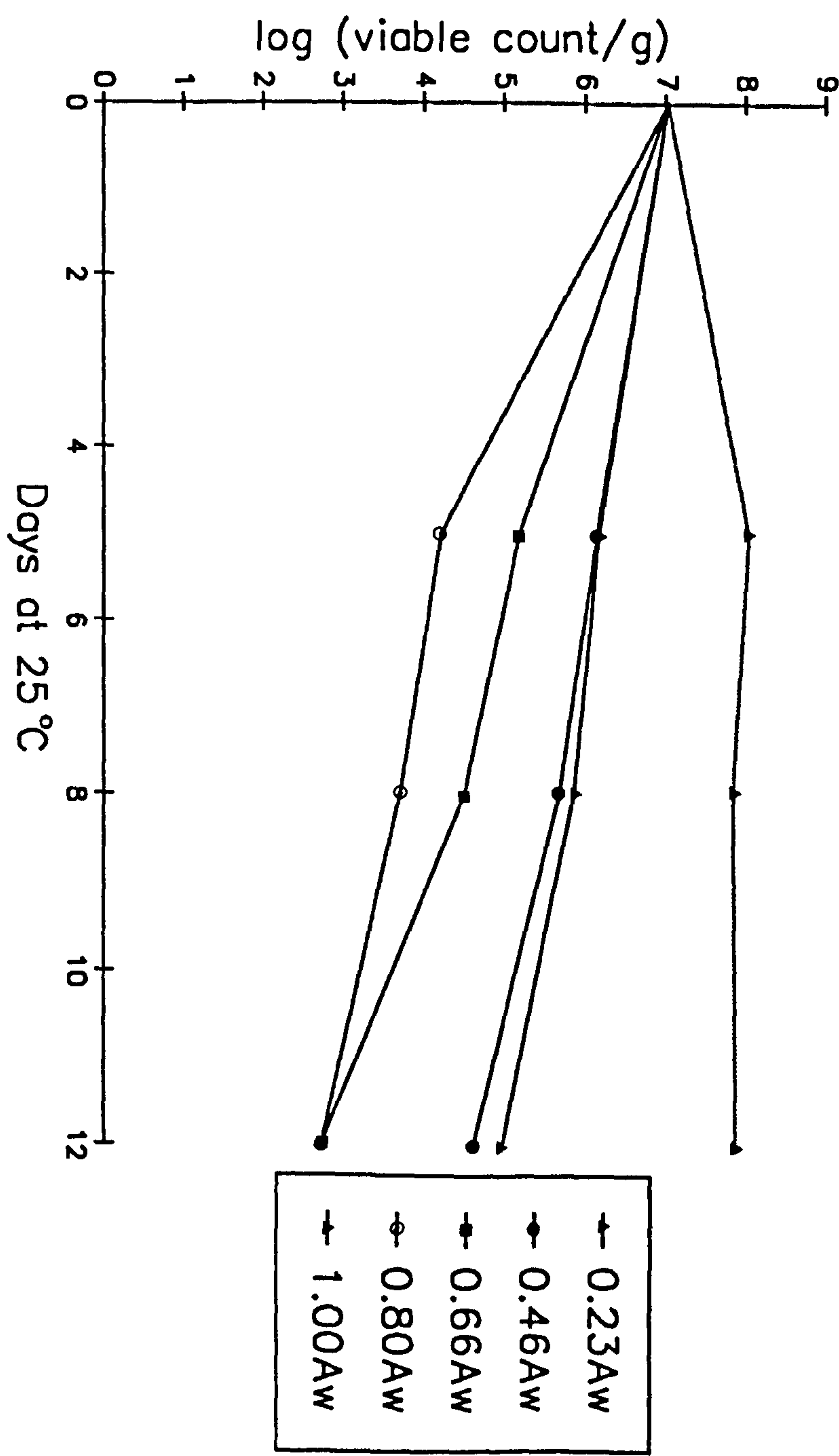


Table 6.11 Viable counts (cfu/g) and [log viable counts] for Lactose tablets with Ent. cloacae

Days at 25°C	Aw of storage				
	0.23	0.46	0.66	0.80	1.00
0	1.08x10 <sup>7</sup> [7.03]	1.08x10 <sup>7</sup> [7.03]	1.08x10 <sup>7</sup> [7.03]	1.08x10 <sup>7</sup> [7.03]	1.08x10 <sup>7</sup> [7.03]
5	1.53x10 <sup>6</sup> [6.18]	1.40x10 <sup>6</sup> [6.15]	1.54x10 <sup>5</sup> [5.19]	1.63x10 <sup>4</sup> [4.21]	1.14x10 <sup>8</sup> [8.05]
8	7.45x10 <sup>5</sup> [5.87]	4.88x10 <sup>5</sup> [5.69]	3.25x10 <sup>4</sup> [4.51]	< 5x10 <sup>3</sup> [<3.7]	7.26x10 <sup>7</sup> [7.86]
12	9.29x10 <sup>4</sup> [4.97]	3.96x10 <sup>4</sup> [4.60]	< 5x10 <sup>2</sup> [<2.7]	< 5x10 <sup>2</sup> [<2.7]	7.77x10 <sup>7</sup> [7.89]

Tablets compressed at 3.65 kN (42.1 MPa).



Figure 6.10 Patterns of survival for Staph. aureus contaminating lactose tablets in a range of relative humidities, at 25°C

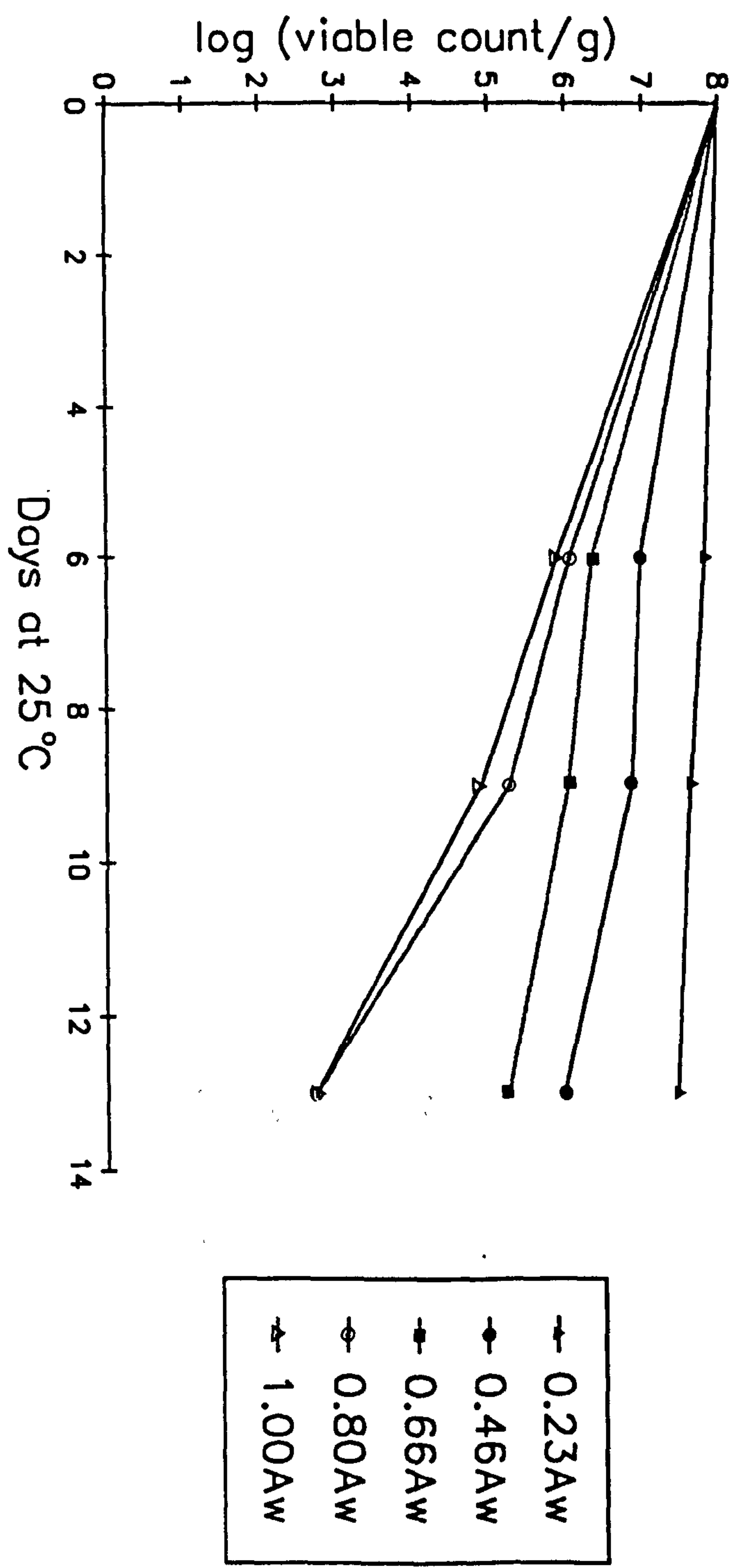


Table 6.12 Viable counts (cfu/g) and [log viable counts] for Lactose tablets with Staph. aureus

Days at 25°C	Aw of storage				
	0.23	0.46	0.66	0.80	1.00
0	9.67x10 <sup>7</sup> [7.99]	9.67x10 <sup>7</sup> [7.99]	9.67x10 <sup>7</sup> [7.99]	9.67x10 <sup>7</sup> [7.99]	9.67x10 <sup>7</sup> [7.99]
6	6.55x10 <sup>7</sup> [7.82]	9.55x10 <sup>6</sup> [6.98]	7.63x10 <sup>5</sup> [5.88]	1.13x10 <sup>6</sup> [6.05]	2.17x10 <sup>6</sup> [6.34]
9	4.31x10 <sup>7</sup> [7.63]	7.30x10 <sup>6</sup> [6.86]	7.51x10 <sup>4</sup> [4.88]	1.79x10 <sup>5</sup> [5.25]	1.07x10 <sup>6</sup> [6.03]
13	2.82x10 <sup>7</sup> [7.45]	9.15x10 <sup>5</sup> [5.96]	< 5x10 <sup>2</sup> [<2.7]	< 5x10 <sup>2</sup> [<2.7]	1.99x10 <sup>5</sup> [5.23]

Tablets compressed at 3.69 kN (42.6 MPa).

Figure 6.11 Patterns of survival for Ent. cloacae contaminating lactose tablets in a range of high relative humidities, at 25°C

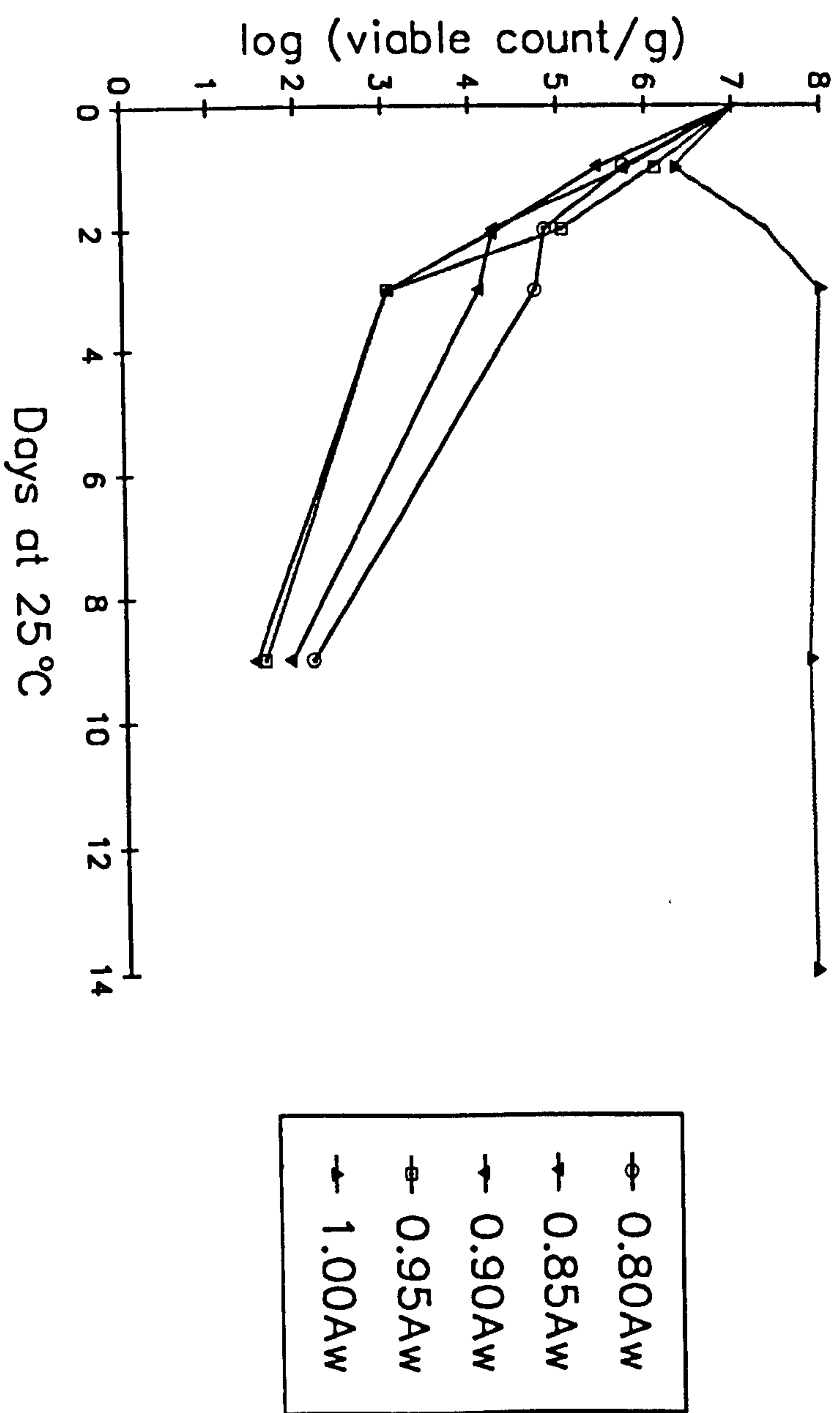




Table 6.13 Viable counts (cfu/g) and [log viable counts] for Lactose tablets with Ent. cloacae at high Aw.

Days at 25°C	Aw of storage				
	0.80	0.85	0.90	0.95	1.00
0	1.15x10 <sup>7</sup> [7.06]	1.15x10 <sup>7</sup> [7.06]	1.15x10 <sup>7</sup> [7.06]	1.15x10 <sup>7</sup> [7.06]	1.15x10 <sup>7</sup> [7.06]
1	5.72x10 <sup>5</sup> [5.76]	2.90x10 <sup>5</sup> [5.46]	6.41x10 <sup>5</sup> [5.81]	1.26x10 <sup>6</sup> [6.10]	2.12x10 <sup>6</sup> [6.33]
2	7.11x10 <sup>4</sup> [4.85]	2.08x10 <sup>4</sup> [4.32]	1.68x10 <sup>4</sup> [4.26]	1.09x10 <sup>5</sup> [5.04]	2.40x10 <sup>7</sup> [7.38]
3	5.34x10 <sup>4</sup> [4.73]	1.10x10 <sup>3</sup> [3.04]	1.23x10 <sup>4</sup> [4.09]	1.10x10 <sup>3</sup> [3.04]	9.71x10 <sup>7</sup> [7.99]
9	1.41x10 <sup>2</sup> [2.15]	< 3x10 <sup>1</sup> [<1.5]	< 8x10 <sup>1</sup> [<1.9]	< 4x10 <sup>1</sup> [<1.6]	-- --
14	-- --	-- --	-- --	-- --	7.85x10 <sup>7</sup> [7.89]

Tablets compressed at 3.65 kN (42.1 MPa).

Figure 6.12 Patterns of survival for Staph. aureus contaminating lactose tablets in a range of high relative humidities, at 25°C

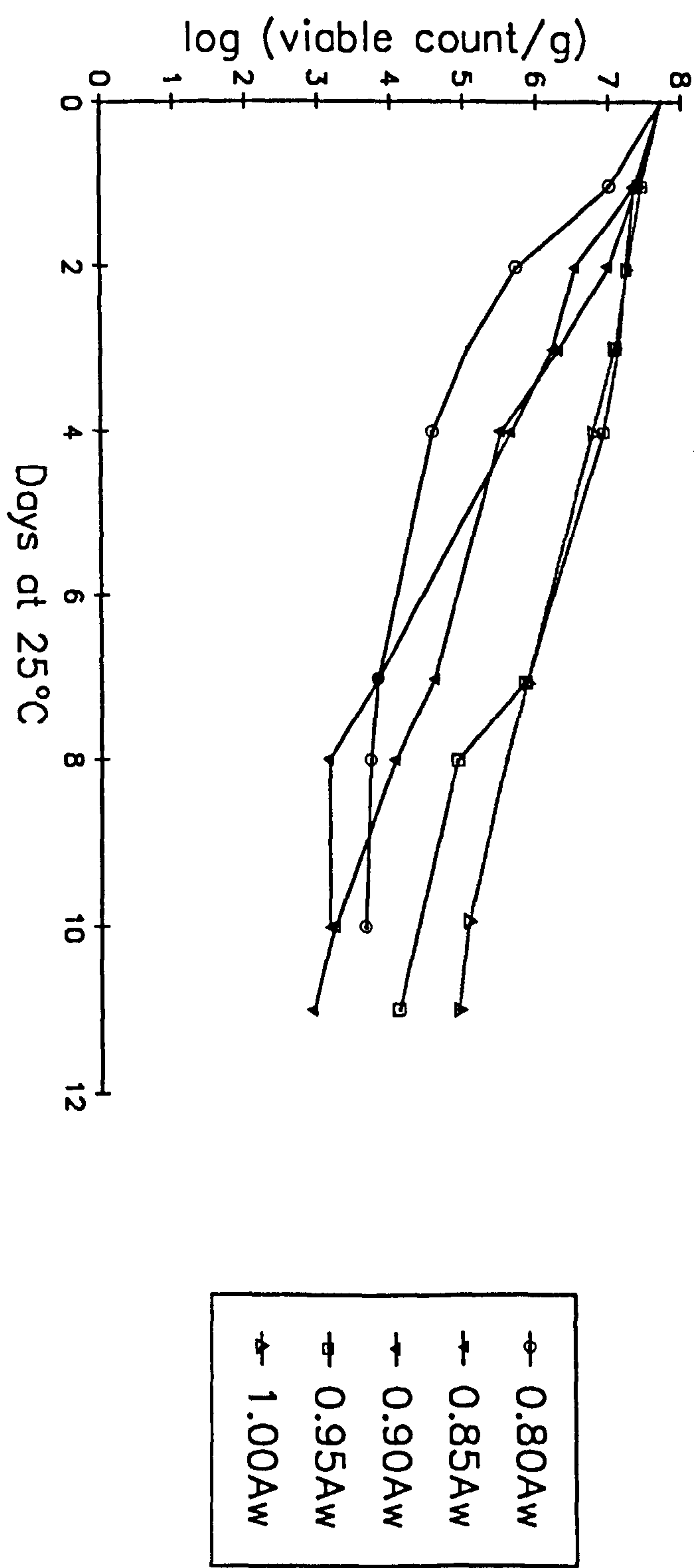


Table 6.14 Viable counts (cfu/g) and [log viable counts] for Lactose tablets with Staph. aureus at high-Aw

Days at 25°C	Aw of storage				
	0.80	0.85	0.90	0.95	1.00
0	5.33x10 <sup>7</sup> [7.73]	5.33x10 <sup>7</sup> [7.73]	5.33x10 <sup>7</sup> [7.73]	5.33x10 <sup>7</sup> [7.73]	5.33x10 <sup>7</sup> [7.73]
1	2.29x10 <sup>7</sup> [7.36]	1.03x10 <sup>7</sup> [7.01]	2.44x10 <sup>7</sup> [7.39]	2.30x10 <sup>7</sup> [7.36]	2.80x10 <sup>7</sup> [7.45]
2	3.46x10 <sup>6</sup> [6.54]	5.36x10 <sup>5</sup> [5.73]	1.00x10 <sup>7</sup> [7.00]	1.77x10 <sup>7</sup> [7.25]	1.85x10 <sup>7</sup> [7.27]
3	1.70x10 <sup>6</sup> [6.23]	1.17x10 <sup>5</sup> [5.07]	2.08x10 <sup>6</sup> [6.32]	1.41x10 <sup>7</sup> [7.15]	1.23x10 <sup>7</sup> [7.09]
4	4.56x10 <sup>5</sup> [5.66]	3.81x10 <sup>4</sup> [4.58]	3.34x10 <sup>5</sup> [5.52]	8.65x10 <sup>6</sup> [6.94]	-- --
7	6.61x10 <sup>3</sup> [3.82]	6.57x10 <sup>3</sup> [3.82]	4.00x10 <sup>4</sup> [4.60]	7.84x10 <sup>5</sup> [5.89]	7.73x10 <sup>5</sup> [5.89]
8	5.14x10 <sup>3</sup> [3.71]	1.39x10 <sup>3</sup> [3.14]	1.13x10 <sup>4</sup> [4.05]	8.30x10 <sup>4</sup> [4.92]	3.93x10 <sup>5</sup> [5.59]
10	4.24x10 <sup>3</sup> [3.63]	1.43x10 <sup>3</sup> [3.15]	1.64x10 <sup>3</sup> [3.21]	-- --	1.16x10 <sup>5</sup> [5.06]
11	-- --	-- --	8.10x10 <sup>2</sup> [2.91]	1.25x10 <sup>4</sup> [4.10]	8.58x10 <sup>4</sup> [4.93]

Tablets compressed at 3.69 kN (42.6 MPa).



Figure 6.13 Patterns of survival for Ent. cloacae contaminating wheat starch tablets in a range of relative humidities, at 25°C

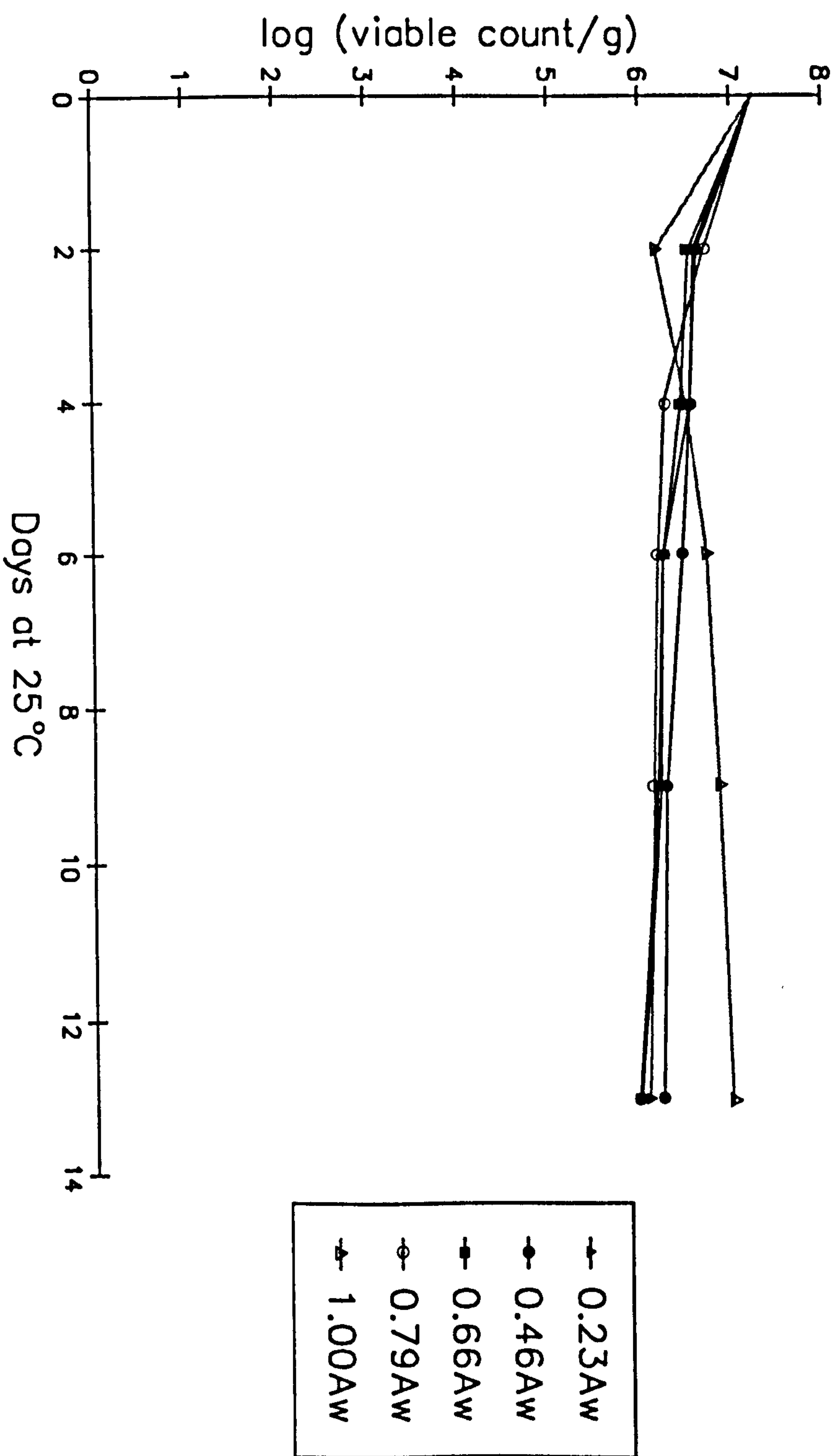


Table 6.15 Viable counts (cfu/g) and [log viable counts] for  
Wheat starch tablets with Ent. cloacae

Days at 25°C	Aw of storage				
	0.23	0.46	0.66	0.79	1.00
0	1.75x10 <sup>7</sup> [7.24]	1.75x10 <sup>7</sup> [7.24]	1.75x10 <sup>7</sup> [7.24]	1.75x10 <sup>7</sup> [7.24]	1.75x10 <sup>7</sup> [7.24]
2	3.96x10 <sup>6</sup> [6.60]	4.27x10 <sup>6</sup> [6.63]	3.45x10 <sup>6</sup> [6.54]	5.17x10 <sup>6</sup> [6.71]	1.52x10 <sup>6</sup> [6.18]
4	3.58x10 <sup>6</sup> [6.55]	3.55x10 <sup>6</sup> [6.55]	2.81x10 <sup>6</sup> [6.45]	1.87x10 <sup>6</sup> [6.27]	3.24x10 <sup>6</sup> [6.51]
6	1.77x10 <sup>6</sup> [6.25]	2.92x10 <sup>6</sup> [6.46]	1.76x10 <sup>6</sup> [6.25]	1.57x10 <sup>6</sup> [6.19]	5.37x10 <sup>6</sup> [6.73]
9	1.50x10 <sup>6</sup> [6.18]	1.90x10 <sup>6</sup> [6.28]	1.67x10 <sup>6</sup> [6.22]	1.39x10 <sup>6</sup> [6.14]	7.08x10 <sup>6</sup> [6.85]
13	9.17x10 <sup>5</sup> [5.96]	1.66x10 <sup>6</sup> [6.22]	9.34x10 <sup>5</sup> 5.97]	1.14x10 <sup>6</sup> [6.06]	9.33x10 <sup>6</sup> [6.97]

Tablets compressed at 4.02 kN (46.42 MPa).

Figure 6.14 Patterns of survival for Ent. cloacae contaminating potato starch tablets in a range of relative humidities, at 25°C

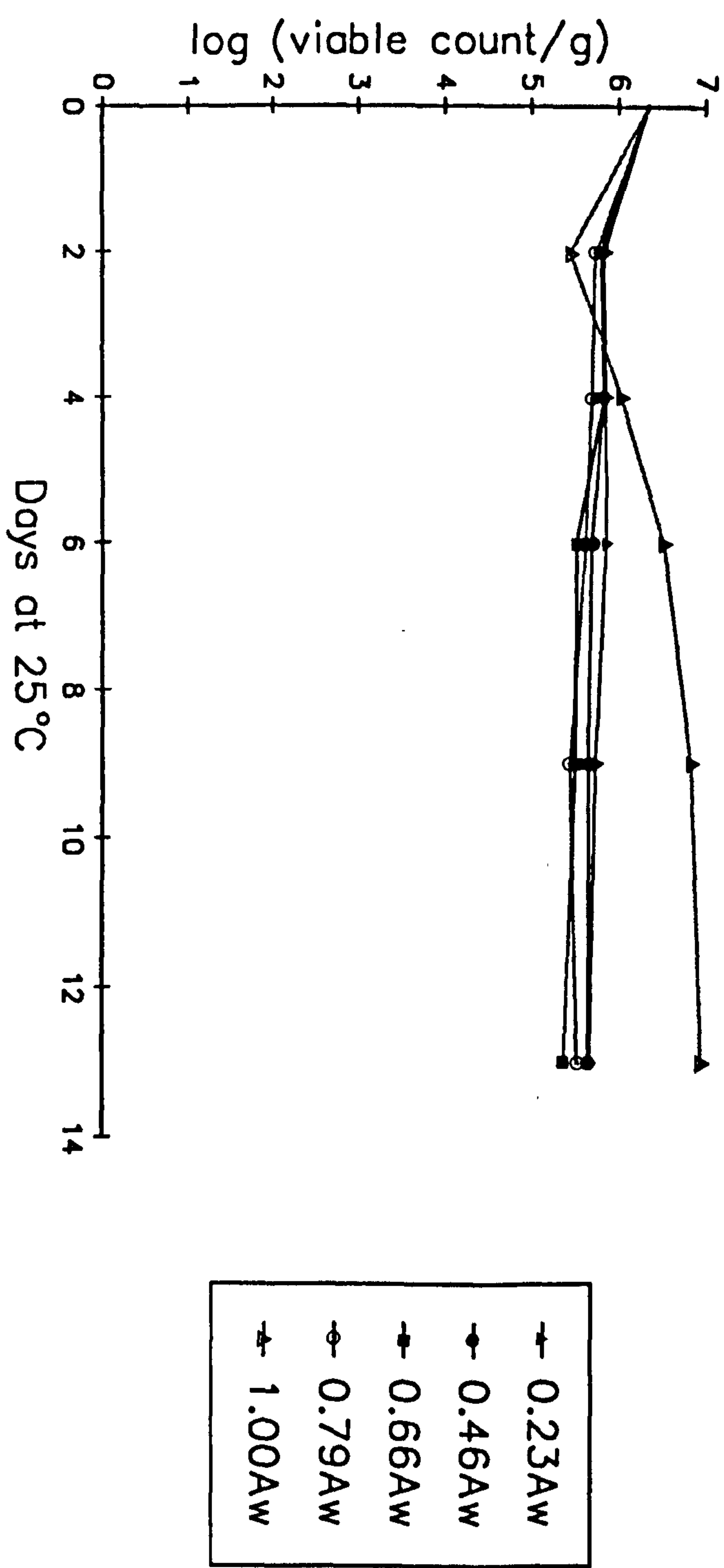




Table 6.16 Viable counts (cfu/g) and [log viable counts] for Potato starch tablets with Ent. cloacae.

Days at 25°C	Aw of storage				
	0.23	0.46	0.66	0.79	1.00
0	2.20x10 <sup>6</sup> [6.34]	2.20x10 <sup>6</sup> [6.34]	2.20x10 <sup>6</sup> [6.34]	2.20x10 <sup>6</sup> [6.34]	2.20x10 <sup>6</sup> [6.34]
2	6.73x10 <sup>5</sup> [5.83]	6.57x10 <sup>5</sup> [5.82]	6.18x10 <sup>5</sup> [5.79]	5.38x10 <sup>5</sup> [5.73]	2.76x10 <sup>5</sup> [5.44]
4	6.68x10 <sup>5</sup> [5.83]	7.12x10 <sup>5</sup> [5.85]	6.82x10 <sup>5</sup> [5.83]	4.90x10 <sup>5</sup> [5.69]	1.09x10 <sup>6</sup> [6.04]
6	3.34x10 <sup>5</sup> [5.52]	7.17x10 <sup>5</sup> [5.86]	4.92x10 <sup>5</sup> [5.69]	4.17x10 <sup>5</sup> [5.62]	3.39x10 <sup>6</sup> [6.53]
9	3.26x10 <sup>5</sup> [5.51]	5.55x10 <sup>5</sup> [5.74]	4.62x10 <sup>5</sup> [5.66]	2.83x10 <sup>5</sup> [5.45]	7.08x10 <sup>6</sup> [6.85]
13	2.43x10 <sup>5</sup> [5.38]	4.79x10 <sup>5</sup> [5.68]	4.55x10 <sup>5</sup> [5.66]	3.44x10 <sup>5</sup> [5.54]	9.33x10 <sup>6</sup> [6.97]

Tablets compressed at 3.73 kN (43.1 MPa).

Figure 6.15 Patterns of survival for Ent. cloacae contaminating wheat starch tablets in a range of high relative humidities, at 25°C

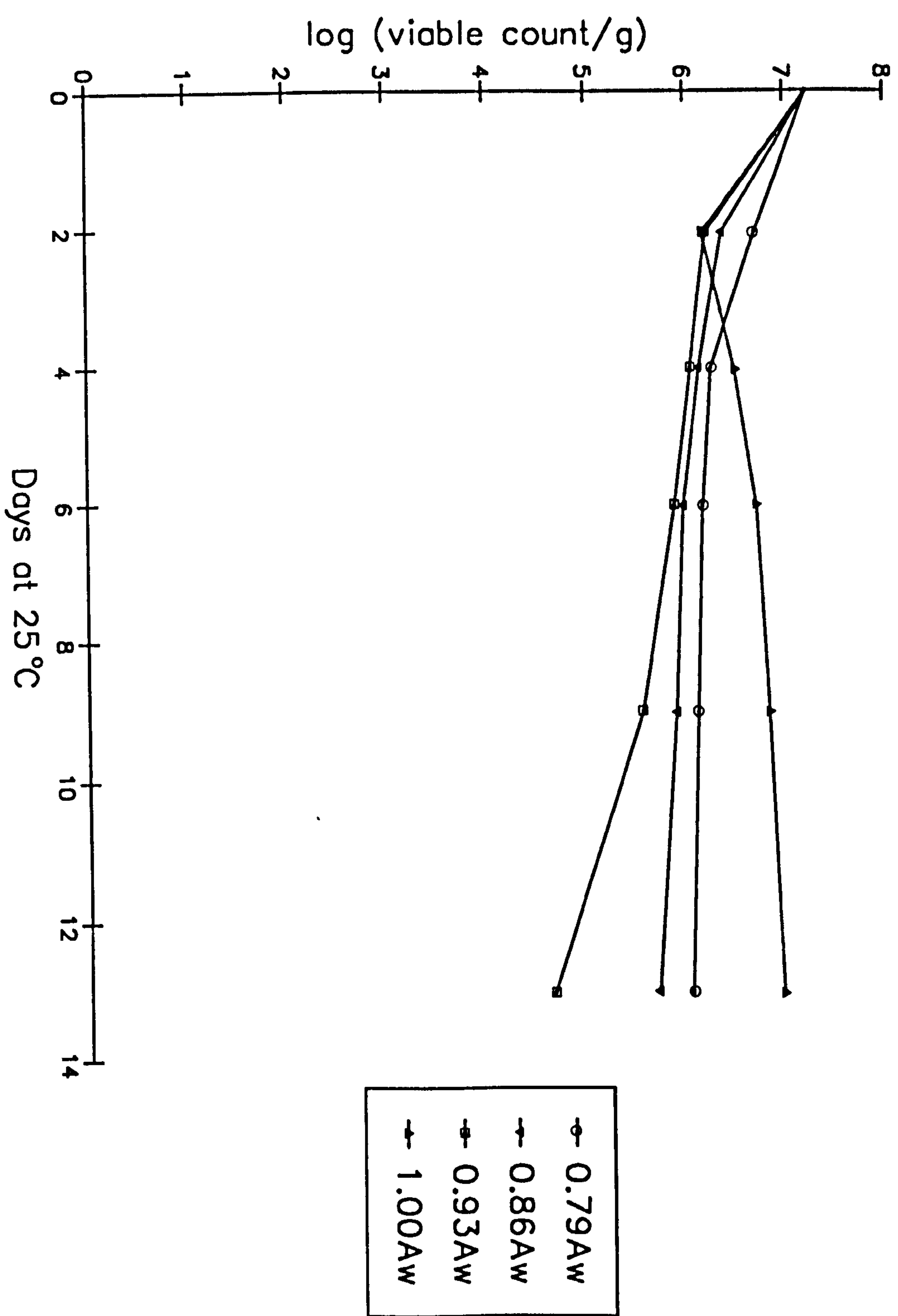


Table 6.17 Viable counts (cfu/g) and [log viable counts] for Wheat starch tablets with Ent. cloacae at high Aw.

Days at 25°C	Aw of storage			
	0.79	0.86	0.93	1.00
0	1.75x10 <sup>7</sup> [7.24]	1.75x10 <sup>7</sup> [7.24]	1.75x10 <sup>7</sup> [7.24]	1.75x10 <sup>7</sup> [7.24]
2	5.17x10 <sup>6</sup> [6.71]	2.40x10 <sup>6</sup> [6.38]	1.66x10 <sup>6</sup> [6.22]	1.52x10 <sup>6</sup> [6.18]
4	1.87x10 <sup>6</sup> [6.27]	1.42x10 <sup>6</sup> [6.15]	1.16x10 <sup>6</sup> [6.06]	3.24x10 <sup>6</sup> [6.51]
6	1.57x10 <sup>6</sup> [6.19]	9.94x10 <sup>5</sup> [5.99]	3.85x10 <sup>5</sup> [5.90]	7.08x10 <sup>6</sup> [6.73]
9	1.39x10 <sup>6</sup> [6.14]	8.38x10 <sup>5</sup> [5.92]	3.85x10 <sup>5</sup> [5.58]	7.08x10 <sup>6</sup> [6.85]
13	1.14x10 <sup>6</sup> [6.06]	5.35x10 <sup>5</sup> [5.73]	4.65x10 <sup>4</sup> [4.67]	9.33x10 <sup>6</sup> [6.97]

Tablets compressed at 4.02 kN (46.42 MPa).



Figure 6.16 Patterns of survival for Ent. cloacae contaminating potato starch tablets in a range of high relative humidities, at 25°C

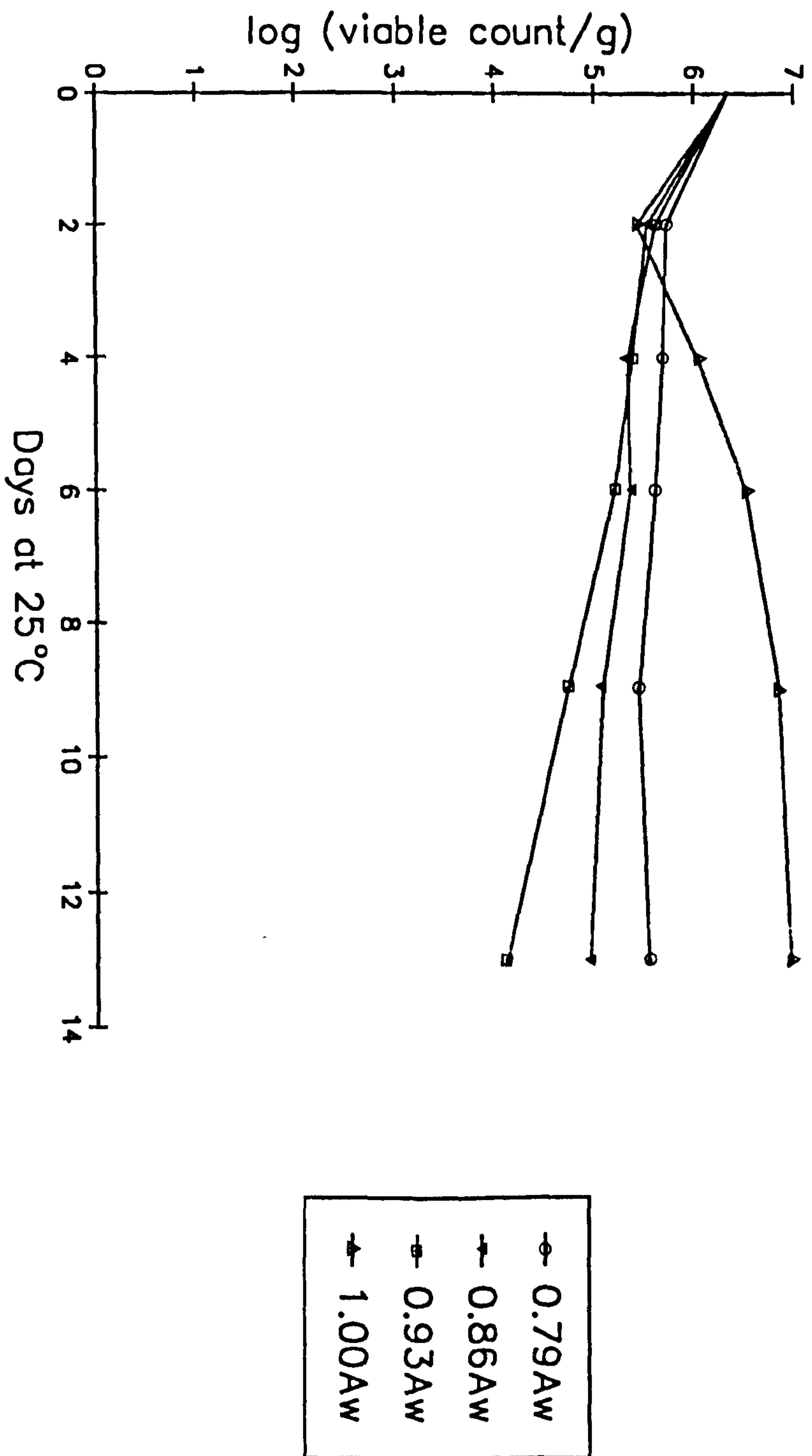


Table 6.18 Viable counts (cfu/g) and [log viable counts] for  
Potato starch tablets with Ent. cloacae at high Aw.

Days at 25°C	Aw of storage			
	0.79	0.86	0.93	1.00
0	2.20x10 <sup>6</sup> [6.34]	2.20x10 <sup>6</sup> [6.34]	2.20x10 <sup>6</sup> [6.34]	2.20x10 <sup>6</sup> [6.34]
2	5.38x10 <sup>5</sup> [5.73]	4.15x10 <sup>5</sup> [5.62]	3.49x10 <sup>5</sup> [5.54]	2.76x10 <sup>5</sup> [5.44]
4	4.90x10 <sup>5</sup> [5.69]	2.19x10 <sup>5</sup> [5.34]	2.44x10 <sup>5</sup> [5.39]	1.09x10 <sup>6</sup> [6.04]
6	4.17x10 <sup>5</sup> [5.62]	2.37x10 <sup>5</sup> [5.37]	1.62x10 <sup>5</sup> [5.21]	3.39x10 <sup>6</sup> [6.53]
9	2.83x10 <sup>5</sup> [5.45]	1.24x10 <sup>5</sup> [5.09]	5.55x10 <sup>4</sup> [4.74]	7.08x10 <sup>6</sup> [6.85]
13	3.44x10 <sup>5</sup> [5.54]	8.97x10 <sup>4</sup> [4.95]	1.28x10 <sup>4</sup> [4.11]	9.33x10 <sup>6</sup> [6.97]

Tablets compressed at 3.73 kN (43.1 MPa).

demonstrates, as previously discussed, that Staph. aureus is more fastidious than Ent. cloacae in its nutrient requirements. For Staph. aureus, it would appear that maize starch provides a more readily-available source of nutrients than Avicel PH101 or lactose.

Although both organisms grew well in powder suspensions with peptone buffer (figures 3.1 and 3.2), the results suggest that residual peptone buffer carried over into the tablets was insufficient to support the growth of Staph. aureus (figures 6.6, and 6.10). However, the residues of the buffer solution were sufficient to support the growth of Ent. cloacae in Avicel PH101 tablets (figure 6.5), when the organism had been unable to multiply in an aqueous suspension of Avicel PH101 alone. Thus at 1.00Aw, the possible growth of micro-organisms with solid excipients will depend very much upon the nature of their nutritional requirements.

Between 0.23 and 0.80Aw, there was insufficient water available for growth of the organisms in any of the tablet formulations. Under these unfavourable conditions, the bacteria survived best at the lower water activities, and the survival generally decreased as the Aw increased up to 0.80Aw. In lactose tablets, each increase in Aw away from 0.23 produced a marked decrease in the viability of both organisms (figures 6.9 and 6.10). This effect was noticeable, although less pronounced, for Staph. aureus contaminating Avicel PH101 tablets (figure 6.6). For the other systems (Staph. aureus in maize starch tablets, Ent. cloacae in



maize starch or Avicel PH101 tablets), survival decreased only slightly between 0.23 and 0.80Aw.

It would seem therefore, that where the moisture conditions for growth were unfavourable, survival was better at the lowest Aw (0.23). Raising the Aw above 0.23 increased the rate of microbial death, in the order lactose > Avicel PH101 > starch.

In order to make more detailed investigations of microbial survival in tablets as the Aw approached 1.00, contaminated tablets were stored at a range of high relative humidities, between 80% and 100% (figures 6.3-6.4, 6.7-6.8, 6.11-6.12). In systems where growth was observed at 1.00Aw, the survival decreased as the Aw increased from 0.80-0.85 towards 1.00, with the organisms losing viability most rapidly at 0.95Aw, which was the highest Aw studied below 1.00. For both organisms, the rate of bacterial death was greatest in lactose or Avicel PH101 tablets, and survival was best in maize starch tablets.

Although the contaminating bacteria grew in the tablets at 1.00Aw where the nutritional conditions were favourable, growth was not observed at any of the Aw below 1.00; this is perhaps surprising where Staph. aureus is concerned, since this organism is known to have a lower water requirement for growth than Ent. cloacae (Troller and Christian, 1978).

The responses of the organisms to Aw varied according to the tablet excipient used. However the results of 6.1.3.2 indicate that maize, wheat and potato starches are not sufficiently

different to give distinctive patterns of survival.

The results of these investigations suggest that in practice, for lactose tablets, vegetative bacteria are only likely to survive at low  $A_w$ . Any increases in the  $A_w$  of the system on storage are likely to result in the death of any contaminating vegetative bacteria within the tablet. On the other hand, such organisms contaminating Avicel PH101 may survive, whilst for the starches, a population of vegetative bacteria should remain viable even at very high  $A_w$  values, where overgrowth by more prolific micro-organisms, such as moulds, is also likely to occur.

Water activities used for tablet storage in these investigations were, for the most part, well below the minimum  $A_w$  values for growth of either organism as reported in the literature. Since growth is not occurring at these water activities, it is unlikely that differences in nutritional value between the powder formulations affect the behaviour of the bacteria on storage, except at the very highest  $A_w$ . However, the water availability to the bacteria at each  $A_w$ , as reflected by the patterns of survival obtained, was characteristic for each excipient used. This suggests that the availability of water to the organisms was different in each tablet system, although the  $A_w$  resulting from storage at each relative humidity was the same.

Since the survival of micro-organisms in different tablet systems did not correlate with  $A_w$ , it must be inferred the availability of water to the organisms is not a simple function of the partial pressure of water vapour, or  $A_w$  as defined. For bacteria in

intimate contact with solids, it would appear that the availability of water to the organisms is also dependent upon the nature of the solid substrate.

Studies of the interactions between the water and the test excipients were undertaken in an attempt to characterize the relative availability of sorbed water to contaminating micro-organisms.



7. THE INTERACTION BETWEEN WATER VAPOUR AND TABLET EXCIPIENTS

## 7.1 METHODS AND RESULTS

### 7.1.1 Introduction

Previous results (section 6) indicate that at a given  $A_w$ , the amount of water sorbed by a contaminated tablet, affects the numbers of surviving bacteria. It had been thought that it was the  $A_w$  alone, and not the moisture content, which would determine microbial survival. A series of investigations were undertaken in an attempt to understand the interaction between sorbed water and the tablet excipients studied.

### 7.1.2 Equilibrium Moisture Content (EMC) and Water Activity

Water-sorption isotherms were constructed for the tablet excipients at 25°C.

#### Method

Constant humidity chambers were prepared using saturated salt or sulphuric acid solutions to control humidity (as previously described in section 6.1.2). Samples were placed in 10ml pyrex beakers to a depth of 5mm for powders, or one layer of tablets, and dried to constant weight overnight in a vacuum oven at 80°C and 10 mm Hg. In order to construct sorption isotherms, samples were transferred to the humidity chambers and left for 8-10 days to equilibrate with the relative humidities at 25°C. To construct desorption isotherms, samples were dried to constant weight as above, and then allowed to equilibrate at 100%RH, before they were transferred to humidity chambers to equilibrate with lower relative humidities at 25°C. After 8 and 10 days in the humidity

chambers, the  $A_w$  of the powder or tablet samples was checked using the Rotronic Hygroskop operating at  $25^{\circ}\text{C}$  as before (section 6.1.2.3), and the samples were accurately weighed using a digital balance (Sartorius type 1412). This operation was carried out as quickly as possible to minimise water vapour transfer with the atmosphere. The percentage EMC was calculated for each storage humidity by comparing the dry weight with the weight after storage for 10 days, by which time it was judged that humidity equilibration had taken place.

### Results

Isotherms of the resultant EMC of the samples as a function of relative humidity at  $25^{\circ}\text{C}$ , are shown in figures 7.1 to 7.9.

Figure 7.1 shows that lactose, maize starch and Avicel PH101 display a range of hygroscopicities. Lactose sorbs negligible amounts of water at most relative humidities, and is not appreciably hygroscopic until 100% RH is approached. The incorporation of magnesium stearate (1% w/w) into lactose tablets raises the EMC. This effect is more marked on the desorption branch of the curve, and results in an enhanced hysteresis effect (compare figures 7.2 and 7.3). The incorporation of magnesium stearate has no significant effect on the EMC of maize starch tablets, in terms of the comparatively large total moisture content achieved with each  $A_w$  (compare figures 7.5 and 7.6). Potato starch is clearly more hygroscopic than maize starch or wheat starch (figure 7.7). Figure 7.8 shows that the three grades of Avicel represented, Avicel PH101, Avicel RC581 and Avicel CL611, have moisture contents modified by the presence of



Figure 7.1

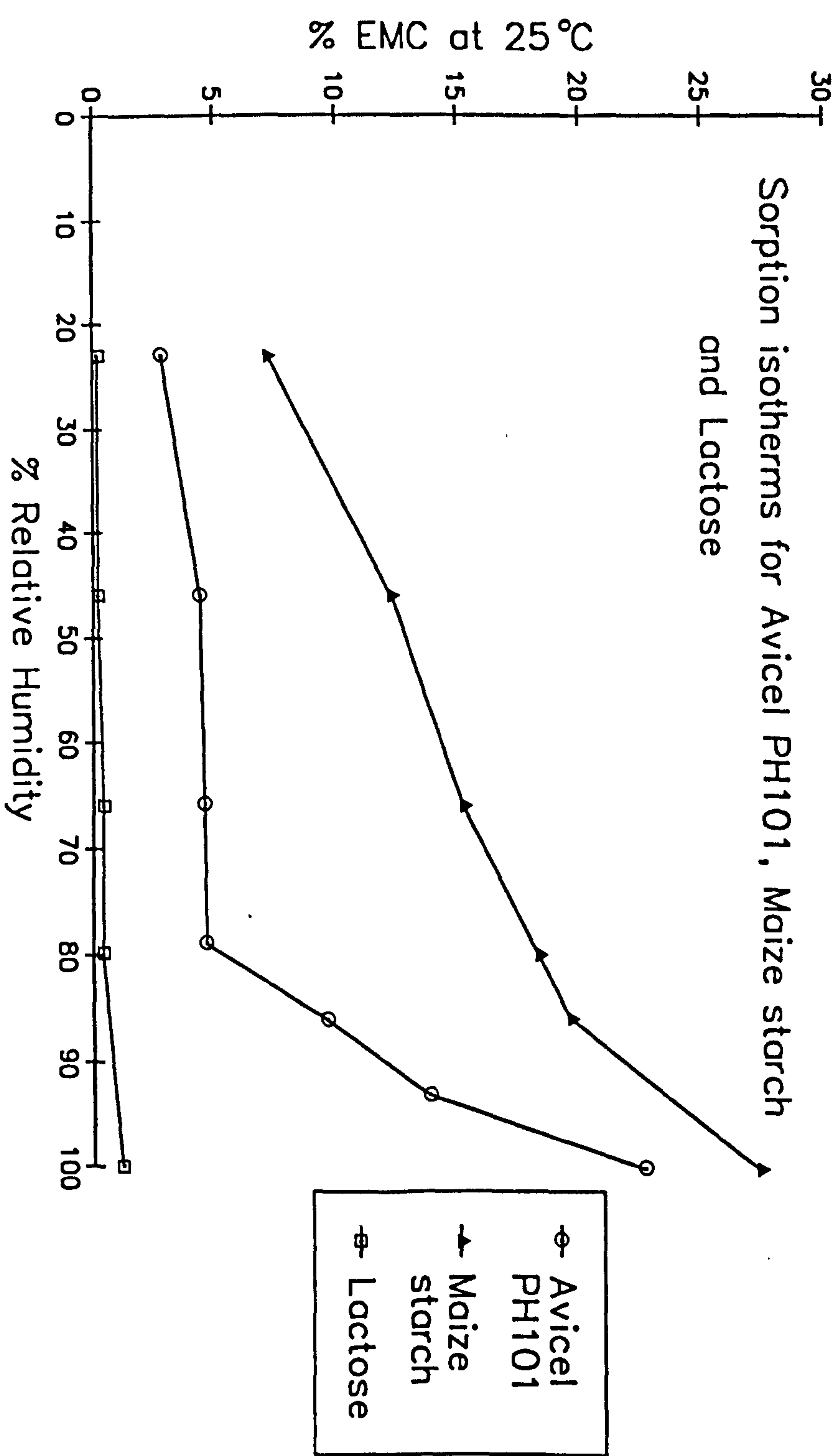


Figure 7.2

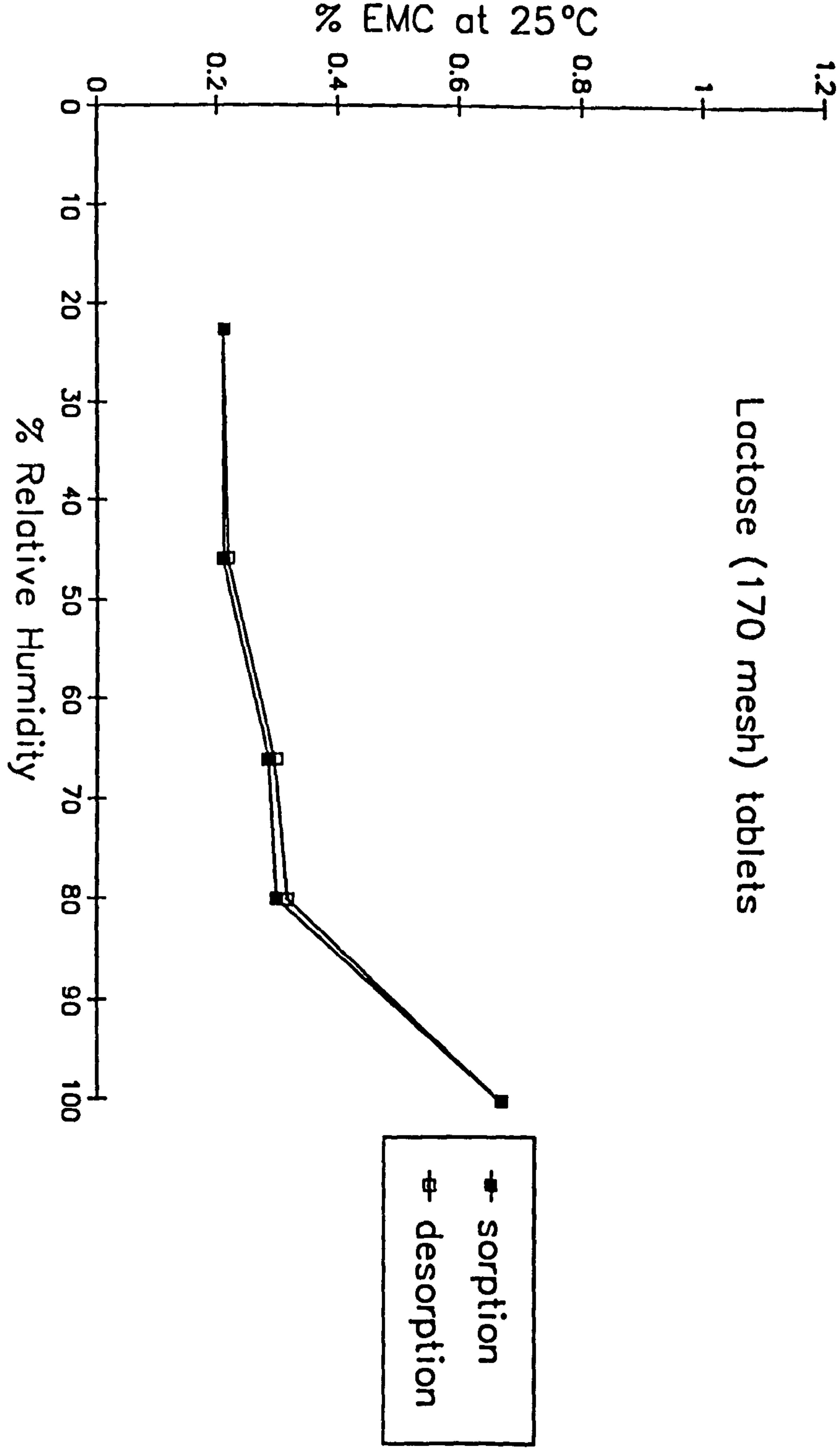


Figure 7.3

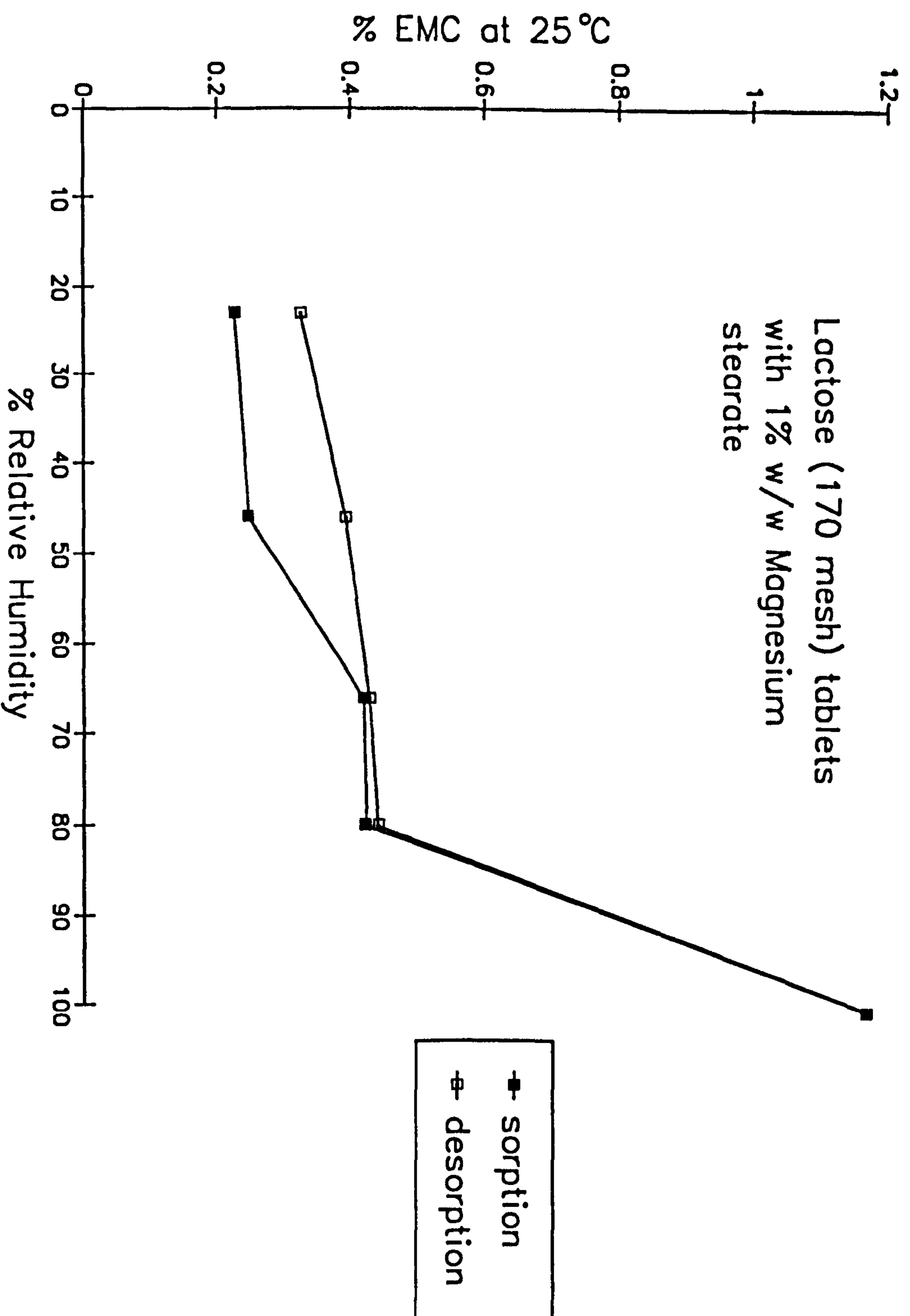




Figure 7.4

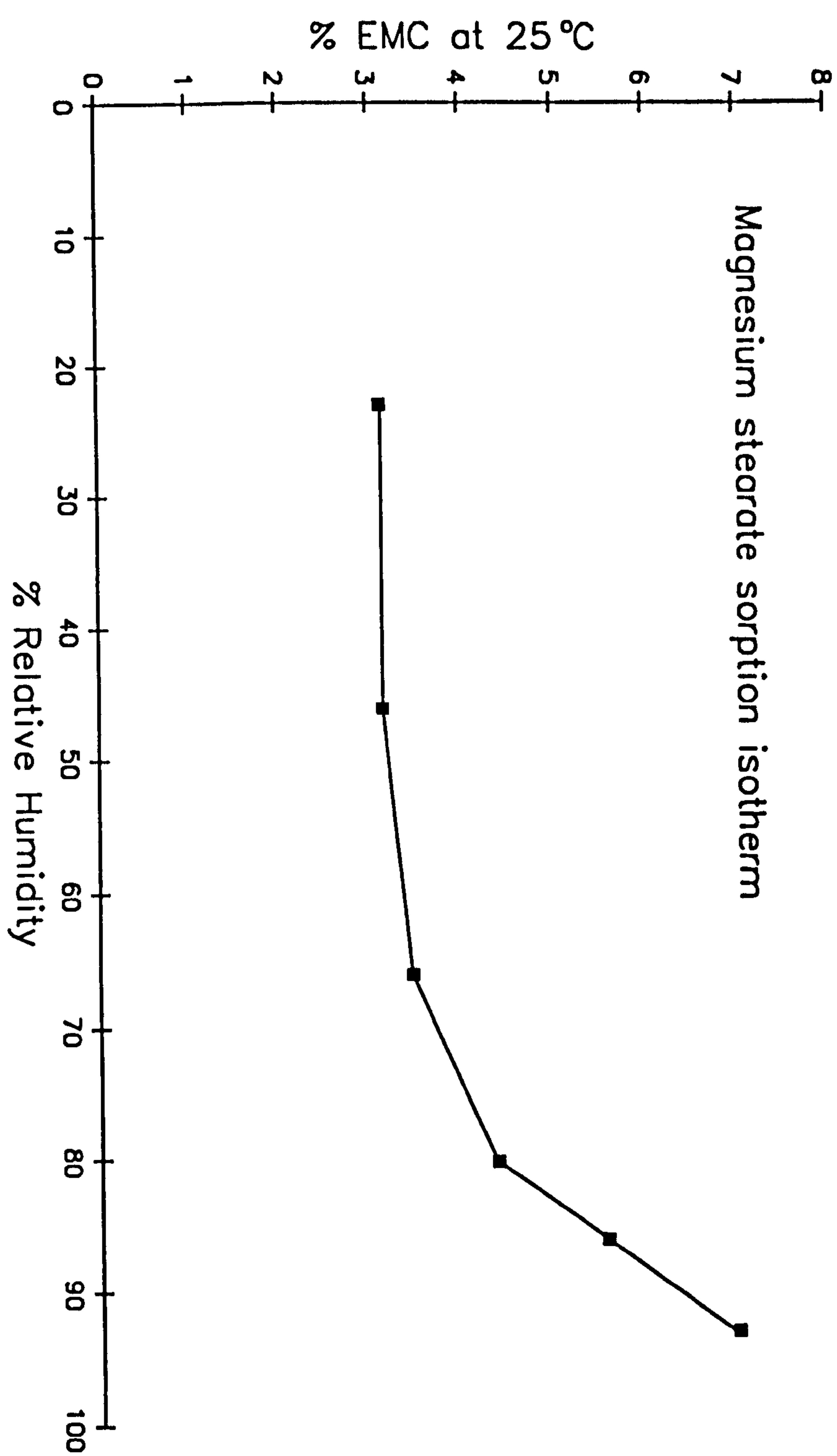


Figure 7.5

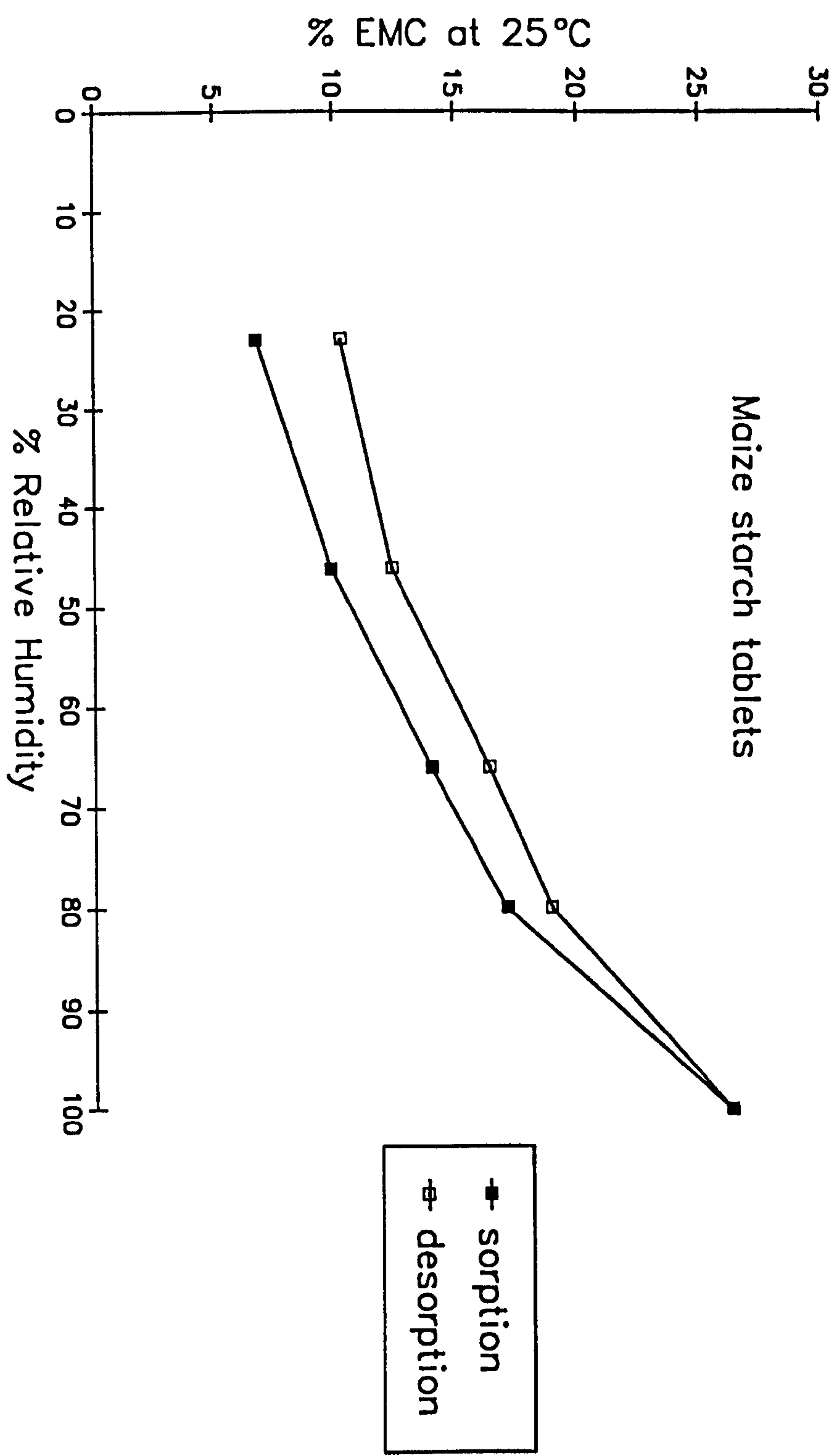


Figure 7.6

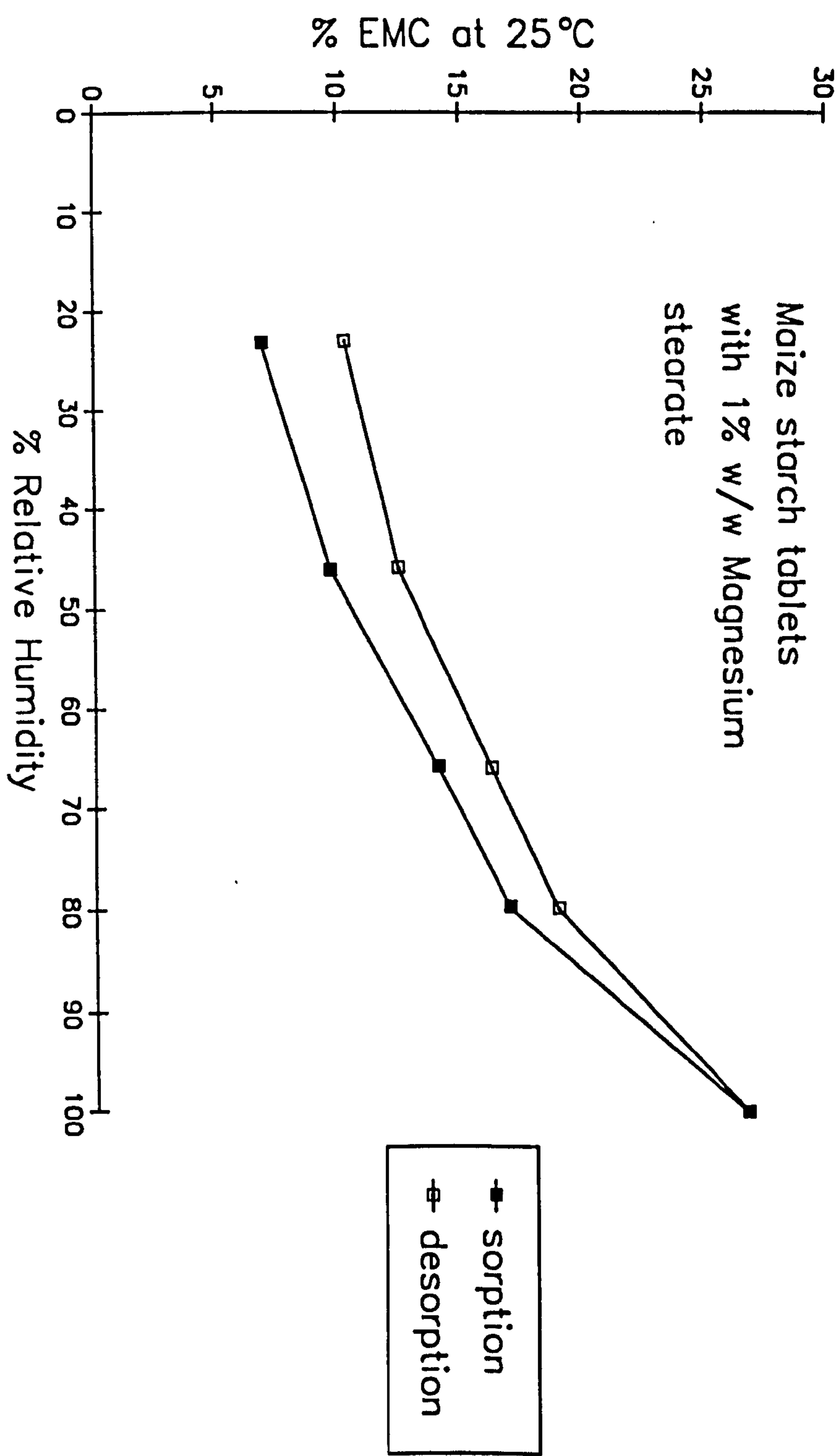




Figure 7.7

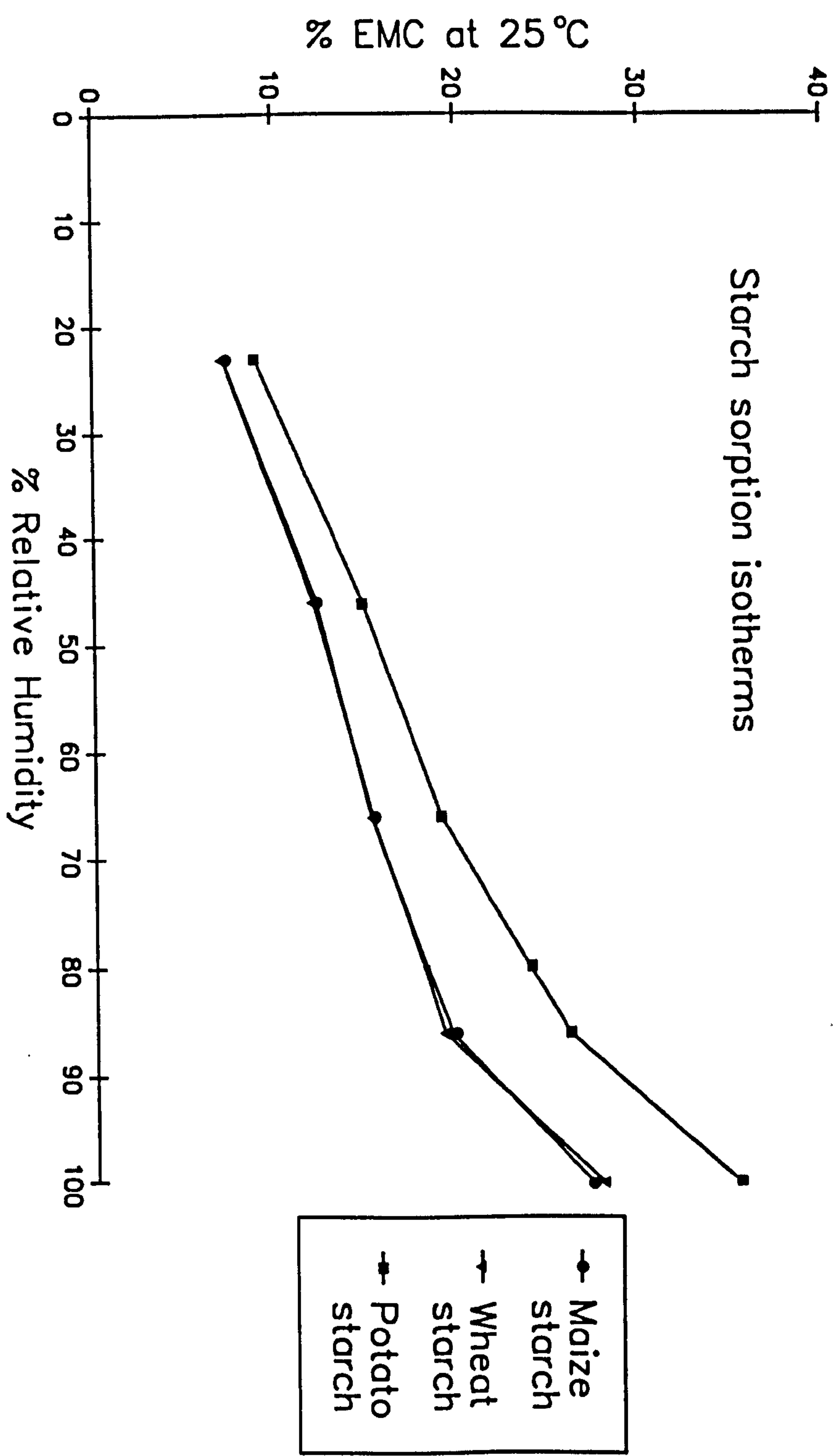


Figure 7.8

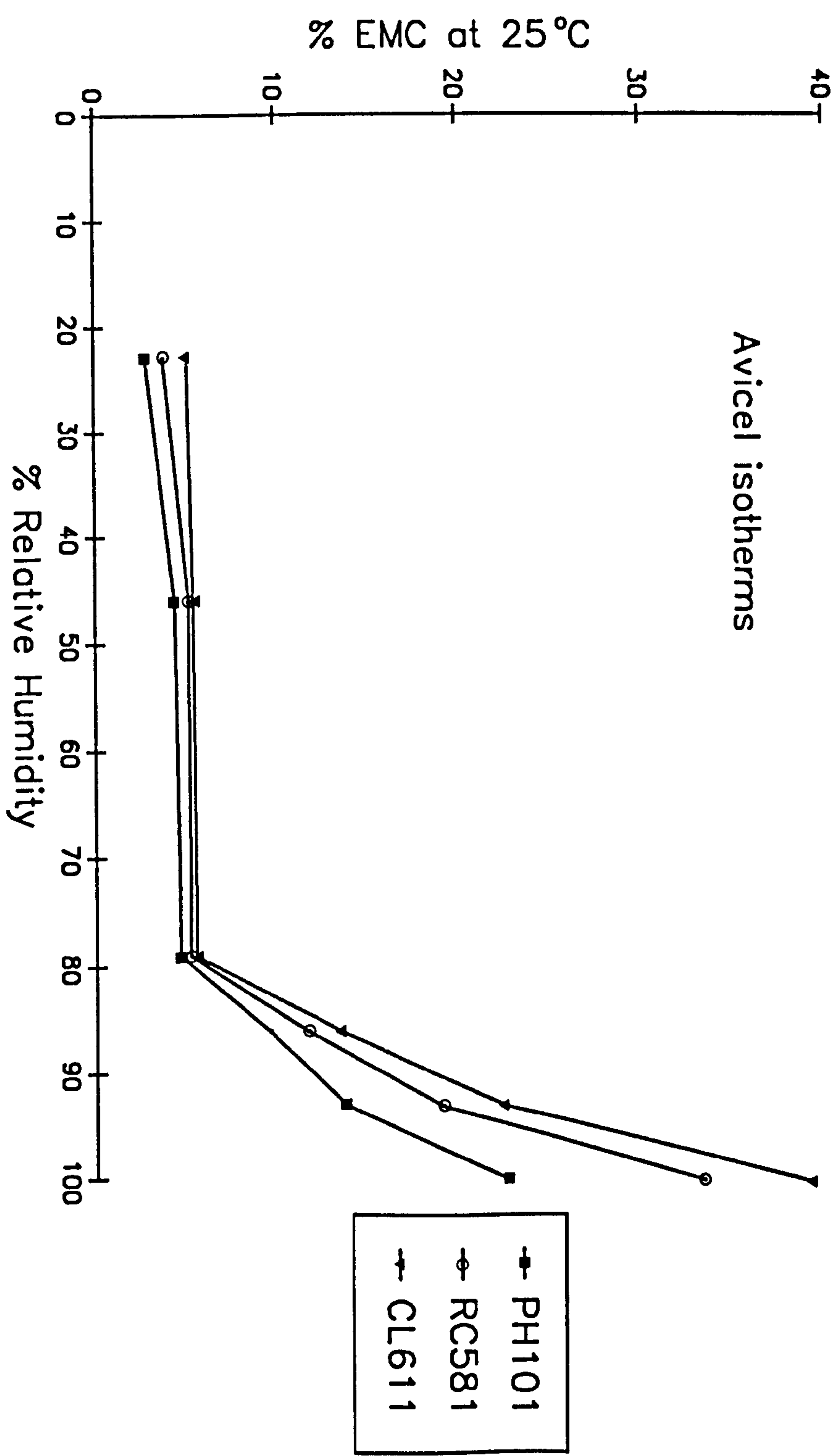
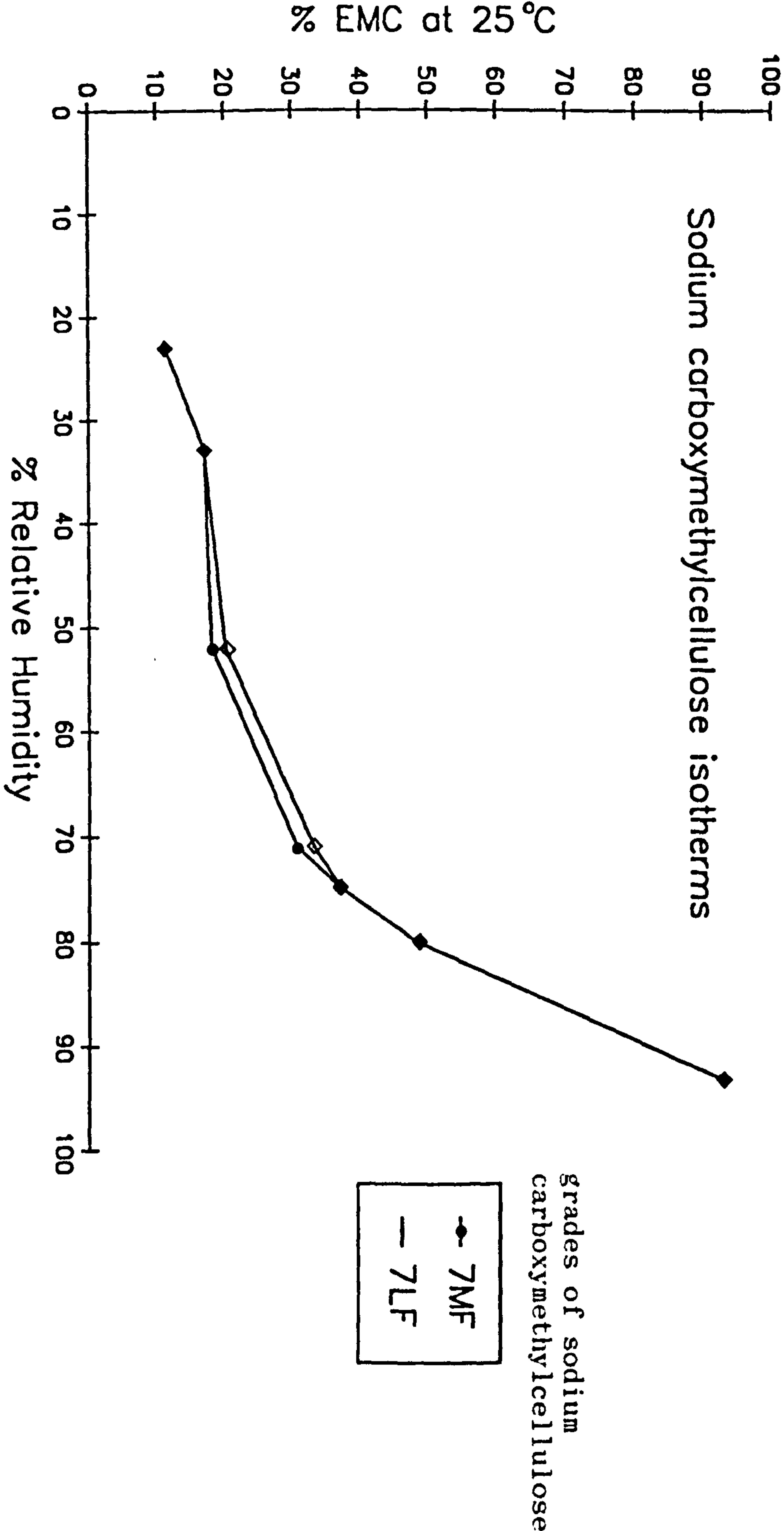


Figure 7.9





increasing amounts of sodium CMC, which is a very hygroscopic material (figure 7.9).

### 7.1.3. Differential Thermal Analysis (DTA)

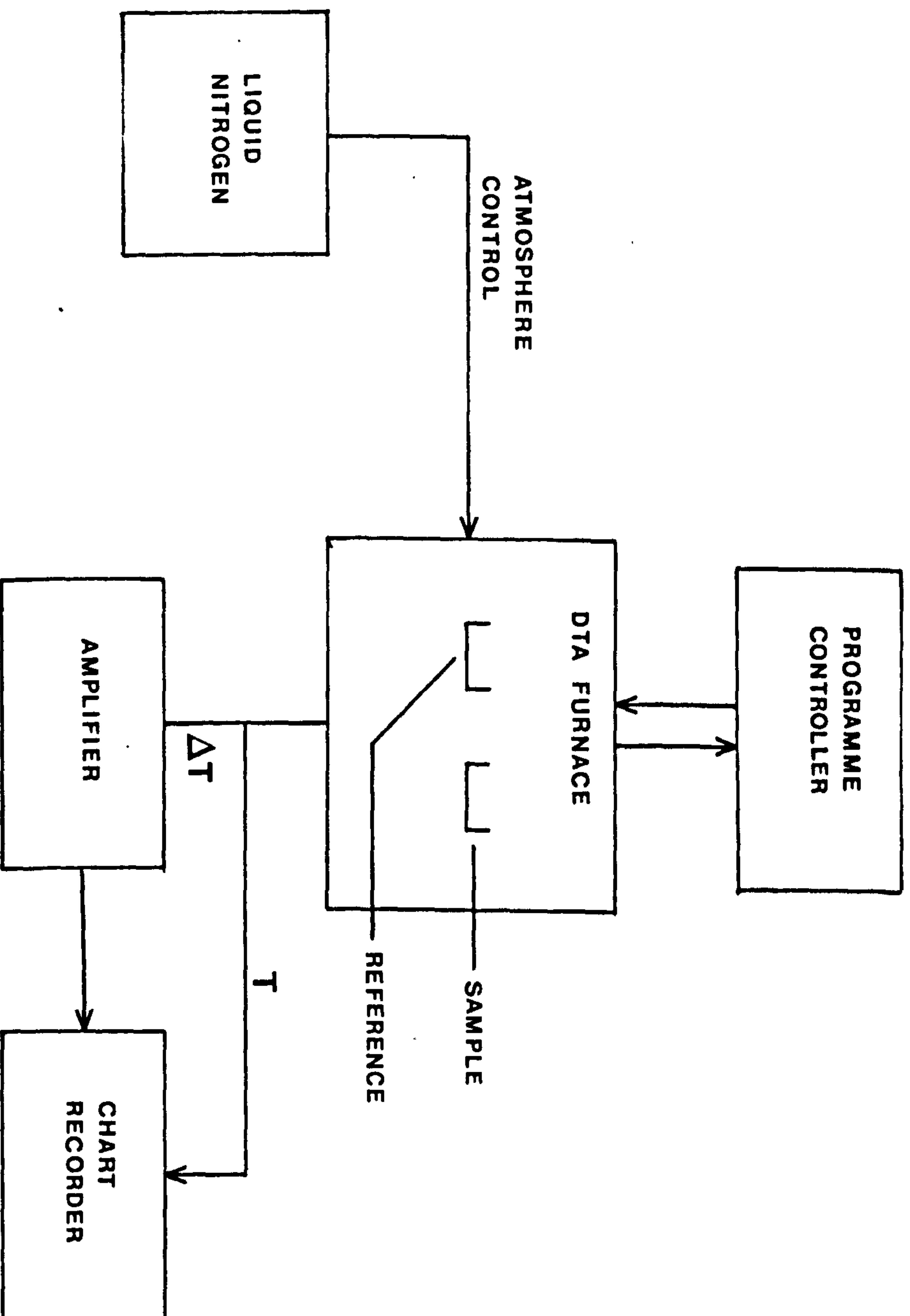
The use of thermal methods to analyse the powder-water interaction were discussed in section 1.4.4. DTA was used to investigate the energetics of the water desorption process for the three starches (maize, wheat and potato starch) and four grades of Avicel (Avicel PH101, RC581, RC591 and CL611, containing a maximum of 0%, 13.8%, 13.8% and 18.8% w/w sodium CMC respectively).

Enthalpies of water vaporization ( $\Delta H_{\text{vap}}$ ) were determined for the tablet excipients as supplied, with a Stanton Redcroft DTA type 671.

#### Method

Figure 7.10 is a schematic diagram of the DTA system. For measurements of energy changes on water vapour desorption, powder samples (about 9mg) were accurately weighed using a digital balance (Sartorius type 1712) into an aluminium pan, placed in the DTA furnace next to an empty aluminium pan as reference. Liquid nitrogen was used to cool the furnace to below 248 K, and the sample and reference were then heated at a constant 10 degrees per minute to about 423 K (150°C). A potentiometric chart recorder (RE 571.20) recorded the thermogram of the temperature difference between sample and reference,  $\Delta T$ , against T. The vaporization of adsorbed water from the powders gave endothermic peaks on the thermograms. The area under each thermogram was measured using an Apple microcomputer graphics package. To calculate the energy

Figure 7.10 Schematic diagram of DTA equipment



represented by the areas under the curves, calibration peaks were obtained by running weighed samples of Indium to melting point. The areas were related to the enthalpy of fusion for Indium (28.38 J/g) for the weight used, giving the number of Joules corresponding to one unit of area on the thermogram. From these data, and the weight loss of the powders on drying (section 7.1.2), it was possible to calculate enthalpies of vaporization for the powder samples.

### Results

Figure 7.11 is typical of the thermograms obtained from starch samples. The moisture contents of the powders and calculated values for the  $\Delta H_{\text{vap}}$  of adsorbed water are given on table 7.1.

The calculated enthalpies of water vaporization for the three starches did not differ significantly ( $P > 0.05$ ), and the values were all around  $60 \text{ kJ mol}^{-1}$  despite the higher EMC of potato starch. Avicel PH101 had a significantly higher ( $P < 0.05$ ) enthalpy of vaporization, at just over  $70 \text{ kJ mol}^{-1}$ , than the starches or other Avicel grades studied. Avicel RC581, Avicel RC591 and Avicel CL611 gave the same  $\Delta H_{\text{vap}}$  values ( $P > 0.05$ ) at around  $50 \text{ kJ mol}^{-1}$ . This figure is lower than that obtained for Avicel PH101 due to the presence of sodium CMC.

#### 7.1.4 The thermodynamic functions for adsorption

##### 7.1.4.1 Vacuum microbalance studies

A gravimetric technique, using a vacuum microbalance, was used to determine the Gibb's function for the adsorption of water vapour



Figure 7.11 Typical DTA thermogram for starch

Heating rate = 10°C/minute  
Chart speed = 5mm/minute

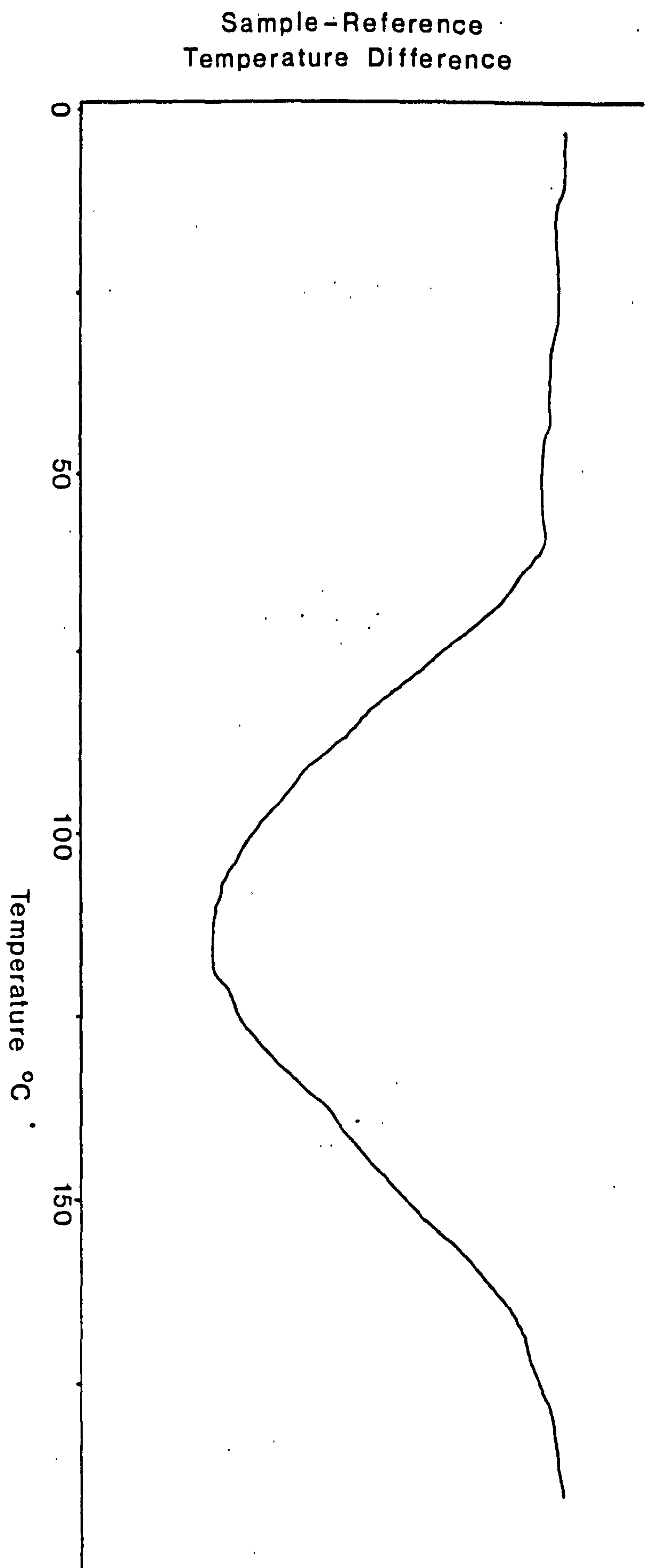


Table 7.1 DTA results

Sample	Moisture content (g/100g)	$\Delta H_{\text{vap}}$ (kJ mol <sup>-1</sup> )	% cv
Maize starch	9.99	61.10 [+/- 4.21]	6.69
Wheat starch	10.00	62.94 [+/- 5.02]	7.97
Potato starch	12.22	59.20 [+/- 0.34]	0.58
Avicel PH101	5.10	70.77 [+/- 5.81]	8.21
Avicel RC581	7.70	47.91 [+/- 3.99]	8.33
Avicel RC591	8.22	52.15 [+/- 4.26]	8.17
Avicel CL611	7.60	49.33 [+/- 4.21]	8.53

onto starch powders (maize starch, wheat starch and potato starch) and Avicel powders (Avicels PH101, RC581 and CL611).

### Method

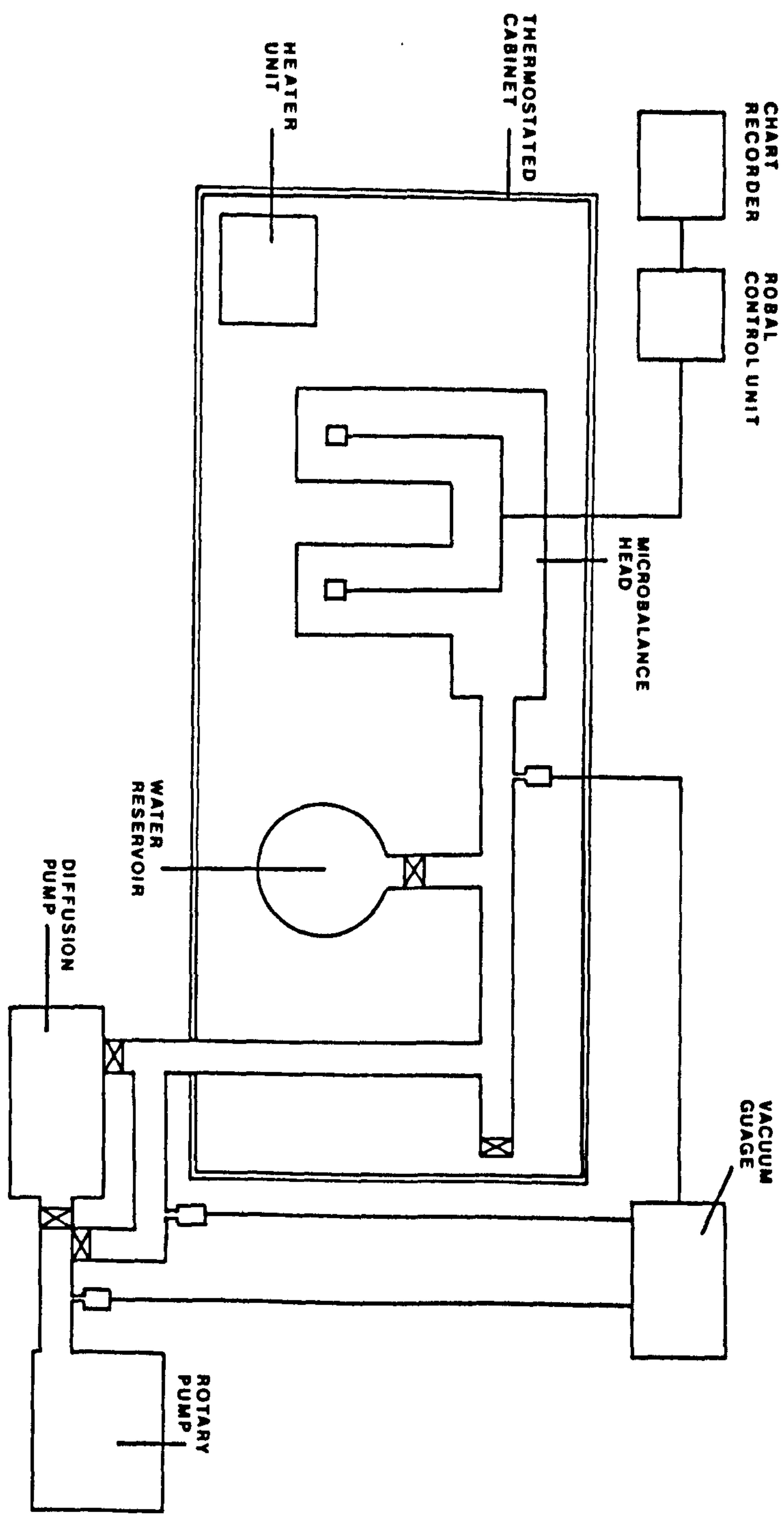
A vacuum microbalance system was used as constructed by Buckton et al (1986), and is represented in figure 7.12. The system consisted of a microbalance with a vacuum head (Robal, CI Electronics), situated in a perspex cabinet. A thermostatically-controlled fan heater maintained the cabinet temperature at 25°C (+/- 0.1 C). A reservoir of outgassed, triple-distilled water was situated close to the balance head and connected via a three-way tap.

Vacuum was achieved by "roughing" the system using an E2M5 rotary pump (Edwards), then switching to a Diffstak 63/150 oil diffusion pump (Edwards) backed with the rotary pump. The "roughing" and backing pressures were monitored by two pirani guage heads (Edwards PRL 10), and the final vacuum achieved by the Diffstak was monitored by a penning guage head (Edwards CP25K). The vacuum guages fed to a digital guage display (Edwards 1005).

For each set of measurements, the Robal was zeroed, and powder sample (about 8mg) was spread thinly onto one balance pan. The system was pumped down to between  $10^{-5}$  and  $10^{-6}$  mbar of vacuum for 18 hours to produce a reproducible, dry surface for subsequent exposure to water vapour. The weight of the dry powder was recorded to 0.001mg. The Robal was tared to zero and the chart recorder for sample weight was set with a range of 2mg. The water reservoir was opened, with the tap simultaneously isolating the



Figure 7.12 Representation of vacuum microbalance equipment



reservoir and balance head from the vacuum. The sample and water vapour were allowed to equilibrate, then the reservoir tap was closed and the system was pumped down to vacuum as before. For each of the powders studied, five replicate sorption determinations were carried out.

#### Calculation of results

Considering the equilibrium:



the equilibrium constant  $K_{ad}$  is derived from:

$$K_{ad} = \frac{[\text{powder with adsorbed water}]}{[\text{powder}] [\text{water}]} \quad (26)$$

If the activity of the powder is taken as unity (according to convention), then the equilibrium constant for the experimental conditions chosen may be calculated from:

$$K_{ad} = [b] / P_o \quad (27)$$

where  $[b]$  = moles of water adsorbed per gram of powder

$P_o$  = saturated vapour pressure of water at the temperature of the experiment.

The Gibb's function for the adsorption of water was calculated from:

$$\Delta G^\ominus = - RT \cdot \ln(K_{ad}) \quad (28)$$

where  $R$  = the gas constant

$T = 298 \text{ K}$

### Results

Table 7.2 shows the calculated values for  $[b]$ ,  $K_{ad}$  and  $\Delta G^\ominus$ . The values given for the Gibb's free energy of adsorption reflect the standard state chosen, and thus are not absolute. The values for  $[b]$  appear to be of a similar magnitude, but t-test comparisons of the results showed that the excipients may be grouped according to the amount of water adsorbed and the corresponding value for  $\Delta G^\ominus$ . Hence water sorption by maize starch and wheat starch was statistically equivalent, but significantly different from water adsorption by potato starch. Data show that the adsorption of water by potato starch was relatively more favoured than adsorption onto maize or wheat starch. Similarly, water vapour adsorption data for Avicels RC581 and CL611 (which are co-processed with sodium CMC) were statistically equivalent, and water adsorption by these excipients was more favoured than by Avicel PH101 (which is microcrystalline cellulose alone).

#### 7.1.4.2 Sorption data from microcalorimetry

An LKB 10700 microcalorimeter was used to measure enthalpies of adsorption of water onto maize starch, potato starch, and Avicels PH101 and CL611.

### Method

The microcalorimeter system used was as described by Buckton and Beezer (1988), and consisted of a calorimeter cell capable of



Table 7.2    Thermodynamic functions from vacuum microbalance studies

Sample	[b] moles water adsorbed/g	% cv	$\Delta K_{ad}$ $\text{mol.g}^{-1}\text{kPa}^{-1}$	$\Delta G^\ominus$ $\text{kJ.mol}^{-1}$
Maize starch	$1.52 \times 10^{-2}$	8.94	$4.79 \times 10^{-3}$	13.23 [+/- 0.22]
Wheat starch	$1.55 \times 10^{-2}$	4.71	$4.89 \times 10^{-3}$	13.18 [+/- 0.12]
Potato starch	$1.98 \times 10^{-2}$	6.14	$6.24 \times 10^{-3}$	12.58 [+/- 0.14]
Avicel PH101	$1.26 \times 10^{-2}$	3.98	$3.98 \times 10^{-3}$	13.69 [+/- 0.09]
Avicel RC581	$1.86 \times 10^{-2}$	2.26	$5.88 \times 10^{-3}$	12.73 [+/- 0.06]
Avicel CL611	$2.19 \times 10^{-2}$	7.09	$6.91 \times 10^{-3}$	12.33 [+/- 0.17]

$R = 8.3143 \text{ J.K}^{-1}.\text{mol}^{-1}$

$T = 298\text{K}$

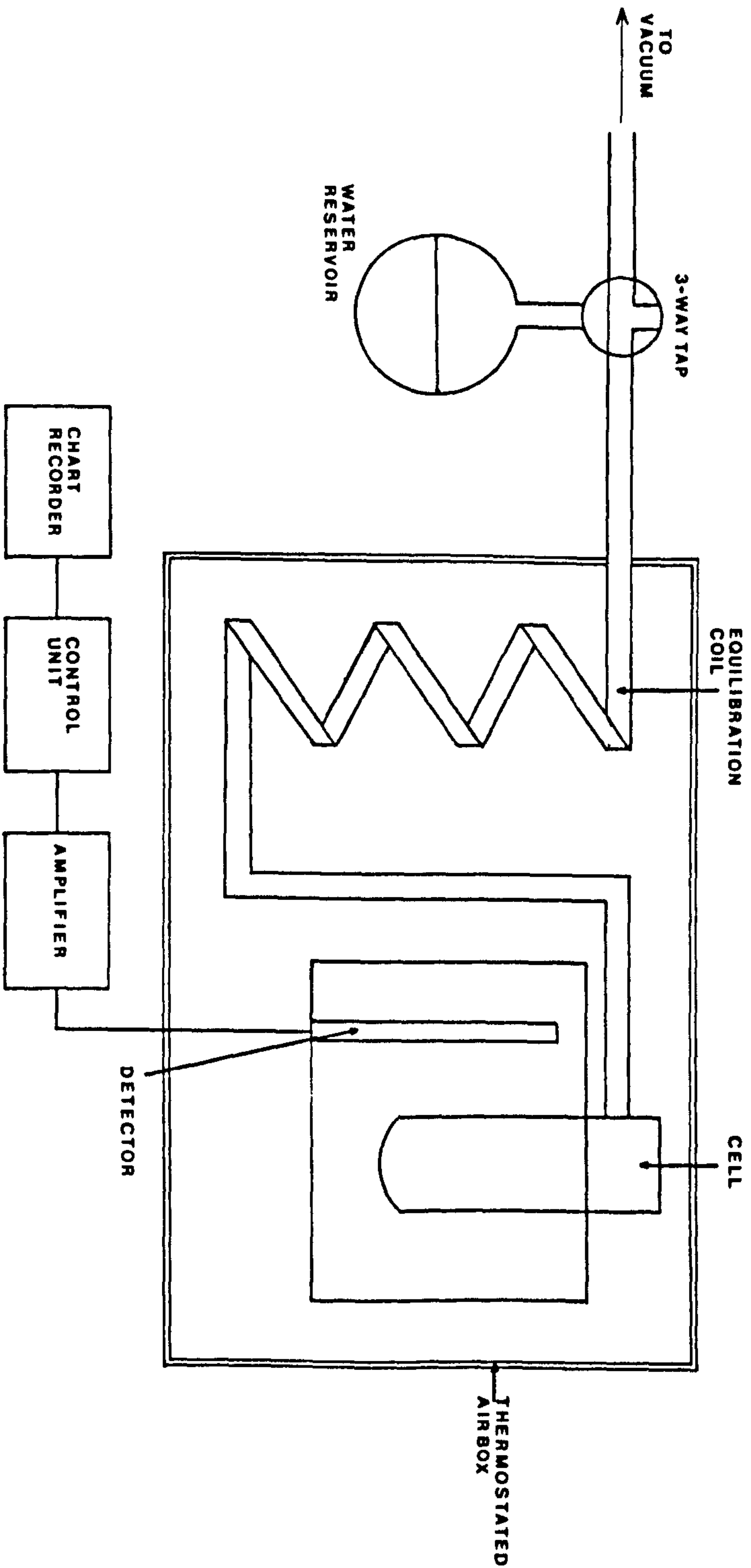
$P_o = 3.1686 \text{ kPa at } 298\text{K}$

detecting a sample temperature change of  $10^{-6}$  °C. The cell was housed in a thermostated container, maintained at 25.0°C (+/-  $10^{-4}$ °C), and connected via a steel tubing system to a vacuum pump, with access to a reservoir of outgassed, triple-distilled water. The apparatus was kept in a temperature controlled room, maintained at 19.0°C (+/- 0.1°C). Figure 7.13 represents the system used.

Powder samples (about 9mg) were accurately weighed into the calorimeter cell. The system was opened to the vacuum pump and left overnight to desorb and "clean" the powder to give a reproducibly desorbed surface, and to equilibrate at 25°C. To allow water vapour sorption, a three-way tap connected to the water reservoir was opened, simultaneously isolating the cell from the vacuum line. Samples typically required 3-4 hours for water adsorption.

Vapour desorption was achieved by reversing this process. The calorimeter signal was drawn as a power-time (p-t) curve on a chart recorder. Calibration was achieved by applying a current of 8mA for 10 seconds from an LKB 2107 microcalorimeter control unit. The heater activated during the calibration process is located in the metal block at the centre of the calorimeter, near to, but not in, the calorimeter cell. There is no absolute confidence that such calibration is truly equivalent to measuring the heat generated within the cell, but the technique is routinely used in the absence of a standard to compare results from different sets of equipment.

Figure 7.13 Representation of microcalorimeter equipment





The areas under the power-time curves were measured using the Apple graphics programme as before (section 7.1.3). The equivalent heat output was calculated by comparison with the calibration peak area, and subtracting the heat generated by the blank (no powder) response.

### Results

Figure 7.14 is typical of the p-t curves obtained. Using data from the vacuum microbalance studies, the area under the p-t curve, representing heat output, was converted to Joules per mole of adsorbed water. This gave values for the enthalpy of adsorption ( $\Delta H_{ad}$ ) of water onto each excipient. Using values for  $\Delta H_{ad}$  and  $\Delta G^\ominus$  previously obtained, the entropy of adsorption ( $\Delta S_{ad}$ ) was calculated from:

$$\Delta G = \Delta H - T\Delta S \quad (29)$$

The results are shown on table 7.3. The measured  $\Delta H_{ad}$  showed that the wetting of potato starch and Avicel CL611 is more enthalpically favoured than maize starch or Avicel PH101.

The decay of the calorimeter signal was analysed for information on the mechanism of powder wetting. Graphs were plotted of the natural logarithm of "power" (i.e.  $dq/dt$ , which is the rate of change of power) as a function of time, showing non-exponential decay of the sample peaks (figure 7.15).

Figure 7.14 Typical microcalorimeter power-time curve

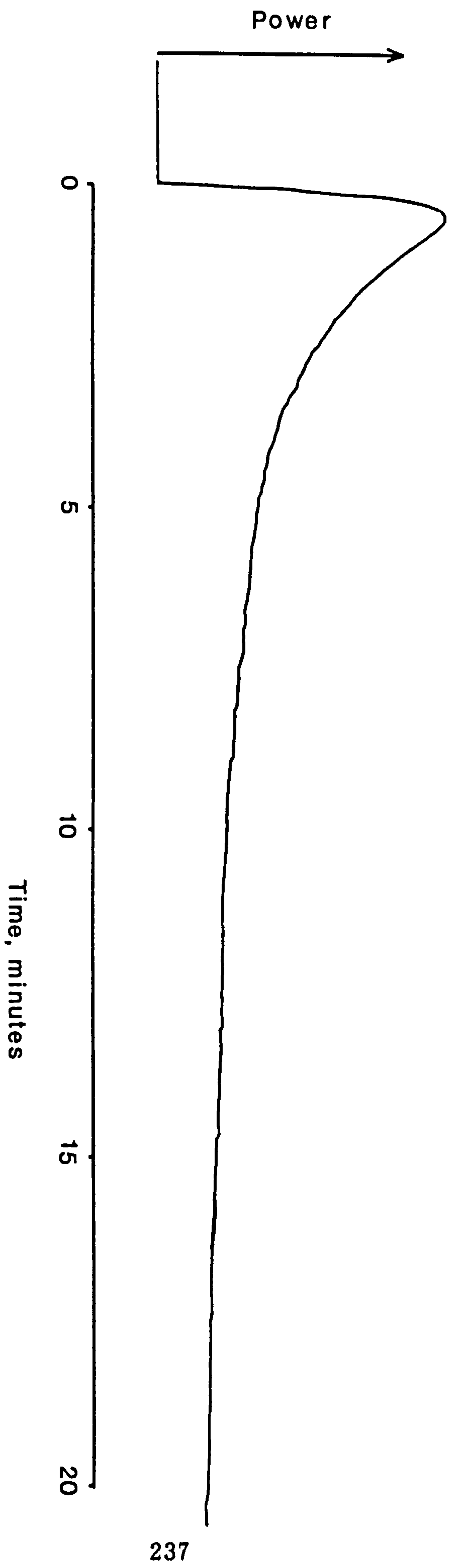


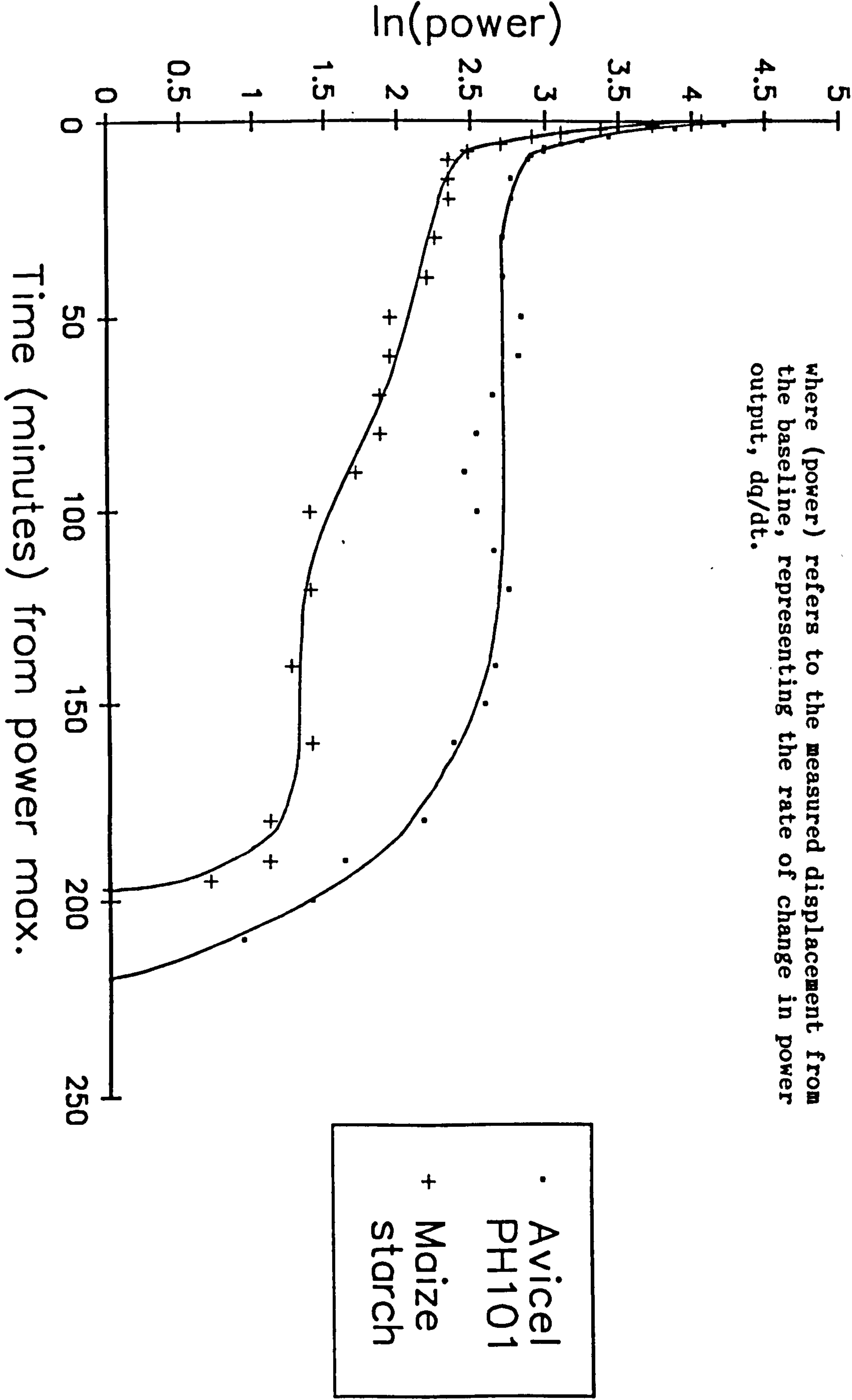
Table 7.3 Thermodynamic functions for adsorption

Sample	Quantity of water adsorbed (mg / gram)	$\Delta G^\ominus$ kJ.mol <sup>-1</sup>	$\Delta H_{ad}$ J.mol <sup>-1</sup>	$\Delta S_{ad}$ J.mol <sup>-1</sup>
Maize starch	273	13.23	-865 [+/- 21]	-47.30
Potato starch	356	13.18	-501 [+/- 16]	-43.89
Avicel PH101	227	13.69	-681 [+/- 14]	-48.22
Avicel CL611	394	12.33	-352 [+/- 5]	-42.56



Figure 7.15 Graph showing the decay of the power-time curve

where (power) refers to the measured displacement from the baseline, representing the rate of change in power output,  $dq/dt$ .



## 7.2 DISCUSSION OF RESULTS

The water vapour sorption isotherms of the tablet excipients (figure 7.1) show that maize starch, Avicel PH101 and lactose have quite different water uptake characteristics.

Across the  $A_w$  range, the EMC of the powders increase in the order lactose < Avicel PH101 < starches. Lactose sorbs comparatively small amounts of water; it is a Class I ("non-hygroscopic") excipient according to the categories defined by Callahan et al (1982), and illustrated in figure 1.1. Avicel PH101 falls into Class II ("slightly hygroscopic"), and the starches into Class III ("moderately hygroscopic"). This ranking corresponds to the increase in stability of microbial populations at different storage humidities. This suggests that contaminating micro-organisms are more stable when attached to solids in more hygroscopic systems.

The excipients used for tableting and storage studies contained 1% w/w magnesium stearate as lubricant. Lactose tablet isotherms (figures 7.2 and 7.3) show that at relative humidities above about 50%, magnesium stearate clearly influences the shape of the isotherm by increasing the quantity of water sorbed. The effect was more noticeable for the desorption branch of the curve, and the net effect was an increase in the hysteresis loop.

It has been shown that magnesium stearate can form a hydrophobic film around solid particles during mixing (Bolhuis et al, 1975), which may retard water desorption. But, a continuous film is not

necessarily a pre-requisite for the lubricant effect of magnesium stearate (Roblot-Treupel and Puisieux, 1986). If film-formation by magnesium stearate retarded water desorption as a rule, some retardation of sorption should also be evident, and an effect of similar magnitude might be expected with other excipients. This is not the case, since isotherms of maize starch tablets, and maize starch tablets with magnesium stearate are practically indistinguishable.

Magnesium stearate, although not particularly hygroscopic, sorbs 10-15 times more water than lactose at the same RH. It is thus more likely that the increase in water sorption by lactose tablets with magnesium stearate, is a direct result of the lubricant raising the potential EMC of the formulation.

Formulation effects which enhance hysteresis, reduce the rate of water vapour desorption from solid excipients. For contaminated wet granulation formulations, a reduction in the rate at which water is lost during granule drying may protect contaminating micro-organisms from some of the harmful effects associated with rapid dehydration.

The tableting process per se does not appear to alter the water-sorbing characteristics of the excipients at the levels of compression used. The isotherm curves for maize starch, and maize starch tablets may be compared (figures 7.5 and 7.6), and are not significantly different. At higher pressures, the effects of compaction on water sorption by the excipients would not necessarily be easy to predict. Studies of powder surface



energetics have shown that compression may produce a deformed "skin" at the tablet surface (Buckton and Newton, 1985), but this does not change the wettability or surface energetics of particles within the powder bed (Luangtana-anan and Fell, 1988). The capillary microstructure may show a shift in pore size distribution towards smaller pores following tableting, but this may be accompanied by an increase in the BET monolayer value if particles undergo fragmentation (Carli et al, 1981).

Water sorption properties of wheat starch, maize starch and potato starch were compared. The isotherms show that potato starch is rather more hygroscopic than the other two starches. It has been established that starches of different origin retain varying quantities of moisture, and early studies proposed that tuber starches such as potato starch have more free cellulose hydroxyl groups available to bond with water than cereal starches (Sair and Fetzer, 1944). Varying amounts of non-cellulosic materials such as proteins, affect the nature of the starch-water interaction by changing the way in which the sorbed water molecules are arranged (Lechert, 1981).

Microcrystalline cellulose (Avicel) may be blended with sodium CMC in a number of different ways, to produce excipients for use as suspending or thickening agents. Sodium CMC is a Class IV, "very hygroscopic" excipient, according to the classification of Callahan et al (1982). Avicel grades with sodium CMC were included in these studies to compare their water-sorbing properties with Avicel PH101.

Avicel CL611 is microcrystalline cellulose co-processed with sodium CMC 7LF (degree of substitution 0.7, low viscosity, food grade), up to 18.8% w/w, and then spray dried. Avicel RC581 is microcrystalline cellulose spray dried with sodium CMC 7MF (degree of substitution 0.7, medium viscosity, food grade) to a maximum of 13.8% w/w. The two grades of sodium CMC both sorb substantial quantities of moisture when exposed to water vapour (figure 7.9). Figure 7.8 shows the sorption isotherms for Avicel PH101, Avicel RC581 and Avicel CL611. Sodium CMC grades of Avicel may sorb double the amount of moisture as Avicel PH101 at the lowest and the highest relative humidities. This shows that incorporating small quantities of comparatively hygroscopic excipients can dramatically alter the EMC of a formulation.

Water sorption isotherms show the amounts of water sorbed by various excipients across the  $A_w$  range. Differential thermal analysis (DTA) was used to quantify the strength of association between water and powders at ambient (35-40%) RH.

DTA was used to determine the enthalpy of vaporization ( $\Delta H_{vap}$ ) for water from the cellulose-based excipient powders. The  $\Delta H_{vap}$  represents the energy required to break hydrogen bonds between water and the cellulose anhydroglucose units. The relative strength of the water-cellulose bond can be deduced by comparison with the  $\Delta H_{vap}$  for pure bulk water, which is about  $41 \text{ kJ mol}^{-1}$ .

The three starches had statistically equivalent ( $P > 0.05$ ) values for  $\Delta H_{vap}$  of about  $60 \text{ kJ mol}^{-1}$ . This shows that the actual



strength of association between water and the starches is quite uniform, despite the larger quantities of moisture sorbed by potato starch than by wheat or maize starch.

The  $\Delta H_{\text{vap}}$  for Avicel PH101 was just over  $70\text{kJ mol}^{-1}$ , at a moisture content of 5.1% w/w. This value fits the data of Khan (1987), who further reported that after the first few percent of sorbed moisture, the enthalpy dropped towards  $50\text{kJ mol}^{-1}$  as the moisture content was increased towards 10% w/w. Thus the Avicel PH101 as supplied contains sorbed water at its most strongly bound.

For the Avicel grades with sodium CMC, the values for  $\Delta H_{\text{vap}}$  obtained were all in the region of  $50\text{kJ mol}^{-1}$ , which is about  $20\text{kJ mol}^{-1}$  lower than for Avicel PH101 at the same RH. Avicel CL611 has a higher proportion of sodium CMC than Avicel RC581 or Avicel RC591, but this in itself did not seem to affect the  $\Delta H_{\text{vap}}$ . Similarly, the different routes by which the sodium CMC grade Avicels are produced did not have a noticeable effect on the  $\Delta H_{\text{vap}}$  obtained. Avicel CL611 and RC591 are produced by co-processing sodium CMC with microcrystalline cellulose, and then spray drying, which results in particles of homogenous appearance, as shown by the scanning electron micrographs (plate 2). Avicel RC581 is produced differently, by spray drying microcrystalline cellulose with sodium CMC. The resultant RC581 particles consist of plaques of sodium CMC and microcrystalline cellulose needles agglomerated together (plate 2). Despite the different methods of production, there was no significant difference ( $P > 0.05$ )



between the enthalpies of vaporization.

Enthalpies of vaporization reveal the relative strengths of association between the various excipients and water, but they do not explain the differences in hygroscopicity between the cellulose-based excipients. For this reason, the thermodynamic functions of adsorption were investigated.

Water adsorption onto the excipient powders was studied firstly by determining the Gibb's functions ( $\Delta G^\ominus$ ) for adsorption with a vacuum microbalance system, and then measuring the enthalpy of adsorption ( $\Delta H_{ad}$ ) directly, using a microcalorimeter system.

The numeric values for  $\Delta G^\ominus$  reflect the standard state chosen for the particular experimental conditions, and thus are not absolute. The data (table 7.2) show that the value for  $\Delta G^\ominus$  for the association of potato starch with water vapour is clearly less than for water vapour with wheat starch or maize starch. Similarly,  $\Delta G^\ominus$  for Avicel is less for the grades produced with sodium CMC than for microcrystalline cellulose alone. Avicel CL611, which contains the highest percentage of sodium CMC gives the lowest  $\Delta G^\ominus$  of all.

The Gibb's function for vapour adsorption is a product of the change in free energy of a surface due to the adsorption of vapour. The vapour-solid interactions which are most favoured energetically, and the relative ease with which water vapour is adsorbed onto the excipient powders, can be deduced by comparison of  $\Delta G^\ominus$  values. Water adsorption is most favoured by potato

starch, and least favoured by Avicel PH101. The ranking of  $\Delta G$  corresponds to the relative hygroscopicities of the excipients as already determined by isotherm studies.

Water adsorption by four excipients - potato starch, maize starch, Avicel PH101 and Avicel CL611 - was investigated further by microcalorimetry. The values for  $\Delta H_{ad}$  were measured directly and the entropy term for the vapour-solid adsorption was calculated with  $\Delta G^\ominus$ . The thermodynamic functions for adsorption are shown on table 7.3.

The values for the entropy term ( $\Delta S_{ad}$ ) show that water vapour adsorption onto maize starch or Avicel PH101 is less entropically favoured than adsorption onto potato starch or Avicel CL611. This means that the two starches and the two Avicels differ in the way that water molecules are adsorbed. Water is adsorbed by maize starch and Avicel PH101 in a more ordered arrangement.

The values for  $\Delta H_{ad}$  were quite distinct for the four different excipients, and ranged between -865 and -352 J mol<sup>-1</sup>. The values were less for potato starch than maize starch, and less for Avicel CL611 than Avicel PH101. The enthalpy are small compared with published data for calorimetric enthalpies of adsorption, which give typical values for  $\Delta H_{ad}$  between -10 and -60 kJ mol<sup>-1</sup> (Schroder, 1984). However, the adsorption enthalpies determined here represent the net energy change for each system. This net energy change is a product of a series of exothermic hydrogen bond formations and endothermic bond breakages, during the swelling of cellulose and the rearrangement of hydrogen bonding water



molecules. These hydrogen bond reactions continue as the powders equilibrate with water vapour to 100% RH. The decline of the p-t curve in each case was not exponential, but a product of the dynamic adsorption process, and apparently in three stages as illustrated by the logarithmic p-t curves for maize starch and Avicel PH101 (figure 7.15). The three phase process may be explained by further consideration of the way water is sorbed by starches and cellulose polymers.

Water adsorption by starches and similar materials has generated a considerable amount of discussion. Gupta and Bhatia (1969) described sorption by starch grains in terms of the behaviour of porous solids; water uptake would be governed by pore geometry and capillary condensation. York (1981) applied the Young and Nelson (1967a) model to water sorption by maize starch, assuming that the starch grains behave as biological cells. Zografis et al (1983) found that specific surface area determinations for starch did not fit those estimated by the Young and Nelson model, because water is sorbed into the starch grain and not just adsorbed at the grain outer surface. Thus Zografis et al (1983) suggested that the Young and Nelson model is not applicable to biological materials such as starch.

Van den Berg et al (1975) concluded that water sorption by starches was almost entirely governed by bond formation with the cellulose anhydroglucose residues. This model presents uptake as a homogenous process, firstly as a monolayer of sorbed water, then as a second layer, and finally water associated with the solid in



a non-specific manner.

Zografí et al (1984) compared data on water adsorption by starches and celluloses including Avicel PH101. They concluded that the amorphous portions of the microcrystalline cellulose are responsible for water adsorption; starches and microcrystalline cellulose take up water by the same basic mechanisms, and uptake is likely to be in the manner previously proposed by Van den Berg et al (1975). This was confirmed for microcrystalline cellulose by the findings of Sadeghnejad et al (1985).

The model described by Zografí et al (1984) was found to fit desorption data for microcrystalline cellulose by Khan (1987). The three phases of power output during direct microcalorimetric measurements of moisture sorption (figure 7.15) strongly support the model. According to Zografí et al (1984), moisture sorption involves initially one water molecule binding to two different anhydroglucose units, and then rearranging so that one water molecule is bound tightly to each anhydroglucose unit. This rearrangement involves bond-breakage, and fits the sharp reduction in power output for maize starch and Avicel PH101, shown on the logarithmic p-t curve during the first 20 to 30 seconds of exposure to water vapour (figure 7.15). Zografí et al (1984) further proposed that between 10 and 19% moisture content, a second water molecule attaches to each anhydroglucose unit, with a lower enthalpy of adsorption than the first. This may explain the levelling-off of the p-t curve for Avicel PH101 and maize starch. The values of 10 and 19% water content approximate to the moisture

levels corresponding to stoichiometries of 1:1 and 2:1 water molecules per anhydroglucose unit. Further water sorbs by weaker hydrogen bonds, and the adsorbed molecules acquire the properties of bulk water as the heat of adsorption decreases and gradually approaches zero. This is illustrated by the drop in the last section of the curves in figure 7.15. The p-t curves for Avicel PH101 and maize starch show similar magnitude and duration of the electrical response. Maize starch shows a steeper slope for the second section of the p-t curve than Avicel PH101. This suggests that the contribution towards the net energy change made by weaker hydrogen bonds occurs earlier in the sorption process for maize starch than Avicel PH101.

The rankings of hygroscopicity of the cellulose-based excipients show that the adsorption process is relatively favoured towards some materials. The functions for adsorption show that water adsorption by potato starch is relatively more favoured than for the other starches, despite the similarity of the enthalpies of vaporization, so that potato starch has a relatively higher EMC. Differences in sorptive behaviour between microcrystalline cellulose and sodium CMC, reflect the greater sorptive capacity of sodium CMC, due to its loss of crystallinity (Zograf and Kontny, 1986), and the fact that adsorption by sodium CMC is more energetically and entropically favoured.

## 8. GENERAL DISCUSSION



Microbial contamination in pharmaceuticals may be hazardous due to the risk of patient infection, and physical or chemical spoilage of the product. Despite their dry nature, solid oral dosage forms and their raw materials may be contaminated with viable micro-organisms. The literature (as reviewed in section 1.5) reports a number of studies of micro-organisms processed in solid oral dosage forms. These investigations were concerned mainly with naturally-occurring contaminants in pharmaceutical excipients (Chesworth, 1980), or with contaminants introduced into formulations via the granulating fluid, or by adding spores to dry powders or tablets (Fassihi, 1978; Fassihi and Parker, 1977a; Plumpton, 1982). Xerophilic fungi such as Aspergillus niger and Penicillium spp, and bacterial spores of Bacillus spp, were chosen for these studies because of their ubiquitous nature and ability to survive under dry conditions. However, the current British Pharmacopoeia is more concerned with vegetative pathogens and requires that the raw materials for solid oral dosage forms should be free from specified organisms such as E. coli, Staph. aureus, the Pseudomonads and Salmonellae. In addition, most pharmaceutical manufacturers routinely test all raw materials and finished products to check for the presence of other Gram negative species such as Enterobacter, Klebsiella and Proteus, for example. In previous studies, non spore-forming organisms such as Staph. aureus and E. coli were introduced into tablets during wet granulation, but were inactivated during granule drying (Fassihi and Parker, 1977b). Undoubtedly, granule drying contributes towards achieving acceptable microbial quality in the finished

tablets. Heavily contaminated tablets are unlikely to result from an efficient drying process, in which water is removed at a reasonably fast rate. The Salmonella muenchen isolated from thyroid tablets as reported by Kallings et al (1966b) were probably able to survive granule drying due to low temperatures necessarily employed to maintain the activity of the thyroid extract. The use of directly-compressible formulations, or the development of alternative methods of tablet processing which avoid high temperature drying, give contaminating micro-organisms a greater chance of surviving tablet processing. Thus we should be aware of possible problems associated with the survival of vegetative as well as spore-forming micro-organisms.

In the initial part of this study, a method was developed to produce dry, directly-compressible excipients contaminated with viable vegetative bacteria. It was possible to produce levels of contaminating micro-organisms in the dry excipients which were consistently high, to allow for subsequent investigation of the effects of tablet processing and survival on storage. In addition, this contamination method appeared to encourage the attachment of contaminating micro-organisms to the excipient particles. It is likely that micro-organisms attached to excipients in this way, would be in a condition more closely related to the situation for naturally-contaminated dry excipients than in previous studies.

Subsequent investigations examined the responses of the contaminating micro-organisms to physical stresses during tablet processing and on storage.



Studies of bacterial inactivation during tableting gave some indication of the nature of the forces acting on the organisms as they are compressed within a powder bed, causing a loss in viability. It was found that bacteria contaminating plastically-deforming materials were inactivated to a greater extent at low compaction pressures, than contaminants of fracturing materials. The presence of a lubricating excipient may reduce the killing effect. These results suggest that tablet compression, and in particular the inclusion of a high degree of plastically-deforming components into a tablet formulation, may contribute to the microbial quality assurance of tablets. For example, a raw material complying with a manufacturer's limit of less than 100 viable bacteria per gram, may after tableting show a 4 log unit reduction, and contain less than one organism in ten grams. However, the effectiveness of bacterial inactivation during tableting depends upon the compaction pressure applied, the compaction mechanism of the formulation, and the influence of lubricating excipients.

Tableting studies suggest that it may be possible to determine the compaction mechanism of a tablet formulation, by monitoring the extent of bacterial kill with increasing compaction pressure. It was possible to detect the change from plastic deformation to fracturing during the compaction of contaminated Avicel PH101. This offers an alternative to more conventional methods of investigating compaction mechanisms, such as the use of density measurements to construct Heckel plots.



During ram extrusion, the spatial distribution of inactivated bacteria reflected the different forces acting on the contaminated wet powder mass. It appeared that the organisms experiencing the greatest degree of shear, were more likely to be inactivated than organisms which were subjected to the highest compression forces. It is likely than the same can be said of microbial kill during tablet compaction, and that inactivation during low pressure compaction in plastically-deforming materials is due to the shear stresses evoked.

Under suitable conditions, contaminating bacteria may survive processing into the final dry dosage forms. Increases in the storage humidity might be expected to encourage bacterial growth and lead to spoilage of the product. This is not necessarily the case: the results of these studies indicate that the fate of the contaminating organisms will depend upon a number of factors, namely the formulation, the types of micro-organisms present, and the nature of the  $A_w$ .

At 1.00  $A_w$ , growth of the test bacteria depended on their nutritional requirements being met, but the bacteria were in any case poor competitors with mould growth which developed on the tablet surfaces. At  $A_w$  below 1.00, no bacterial growth occurred during the storage period, even at very high  $A_w$ . This shows that, for growth at  $A_w < 1.00$ , all other conditions, such as temperature and nutrients must be near perfect for the organisms concerned. It is likely that the conditions of storage were were not sufficiently close to the optima for growth of the organisms used.

Thus at  $A_w$  below 1.00, it was not bacterial growth, but bacterial survival that was being monitored. It was found that the extent of survival of the vegetative organisms on storage depended on both the  $A_w$  of the tablet, and the water uptake characteristics of the formulation used. For poorly hygroscopic materials such as lactose, an increase in  $A_w$  might well lead to the death of the contaminating population. However, in the presence of more hygroscopic excipients, the contaminating population is more likely to remain stable despite rises in  $A_w$ . At the lower end of the  $A_w$  range, stored tablets are more stable from a physicochemical aspect, but they are also a better environment for maintaining the viability of contaminating bacteria.

Of the excipients studied, the starches were thought to be most likely to introduce bacterial contaminants into pharmaceutical formulations, since they are natural products, and quite hygroscopic. The starch samples used here - maize, wheat and potato starch - had higher total viable counts before sterilization than any of the other test excipients. The starches supported growth of bacteria in aqueous solutions, and allowed bacterial survival across a wide range of storage humidities in tablets. The literature typically reports higher bacterial counts in potato starch than in wheat or maize starch (Kallings *et al*, 1966b; Chesworth, 1980). Adsorption isotherms showed that potato starch is more hygroscopic than wheat or maize starch. This is not apparent from the enthalpies of water vaporization from the starches, which were practically indistinguishable. The



thermodynamic functions for adsorption indicated that water vapour adsorption onto potato starch is a more favoured process than for the other starches, hence the higher hygroscopicity.

Bacterial survival in stored tablets suggests a link between the hygroscopicity of the excipients and the resilience of bacteria to different relative humidities. At  $A_w$  much below 1.00, we assume that bacterial growth does not occur, and therefore the finite EMC at each  $A_w$  should be of no consequence. The link between hygroscopicity and bacterial survival may be the energy involved in water vapour adsorption. Microcalorimetric measurements showed that prior to equilibration at 100% RH, lower energies are involved in water adsorption to starch than to microcrystalline cellulose. The lower  $\Delta H_{ad}$  may indicate that water adsorbed by starch is relatively more available to adherent bacteria than water adsorbed by microcrystalline cellulose. This may explain why "dry" starches are a comparatively hospitable environment for adherent bacteria, but still leaves us with the dilemma of the reason for bacterial death at high  $A_w$  in lactose.

Since the introduction and adoption of microbial limits and GMP for solid oral dosage forms, it is generally considered that such products do not present a significant microbiological hazard. However, microbiologists must be aware that new developments in the technology of solid dosage form processing could change this situation, as opportunist micro-organisms can flourish in apparently hostile environments. This has been a hard lesson for the food industry, where reports of food-borne listeriosis have



hit the headlines. Human listeriosis had been an obscure disease and received little attention. Listeria monocytogenes is able to grow unchallenged at refrigeration temperatures. Listeria spp are now important pathogens, because of the 46% mortality associated with listeriosis (McLaughlin, 1987). This is a sharp reminder that micro-organisms may find a niche even in extreme environments, and the failure to anticipate situations which may encourage the survival and growth of contaminants could have very serious consequences.

It is important to recognise that certain types of pharmaceutical raw materials are likely to allow the survival of vegetative organisms, even in dry products. Limits applied to non-sterile pharmaceuticals and raw materials have improved the microbial quality of these products over the last two decades, but such limits are set with the assumption that microbial growth will not occur after testing. A change in processing or formulation may increase the potential for bacterial survival and growth in a product. For example, microcrystalline cellulose co-processed with sodium CMC is more likely to support growth than microcrystalline cellulose alone. Raw materials of natural origin require the most careful monitoring, since they may carry the highest microbial load. It appears that bacteria have a better chance of surviving in stored dry products if the formulation is based on hygroscopic materials.

## 9. CONCLUSIONS

- (i) Dry tablet excipients were contaminated with viable vegetative bacteria by first growing the organisms in aqueous powder suspensions. Growth was enhanced by the presence of small quantities of nutrients. Starch suspensions supported bacterial growth, unlike suspensions of insoluble microcrystalline cellulose (Avicel PH101). However, the bacteria grew in suspensions of microcrystalline cellulose co-processed with sodium carboxymethylcellulose (Avicel CL611), which has a lower degree of cellulose substitution.
- (ii) Scanning electron microscopy showed bacterial adhesion to the powder particles. The excipients contaminated by this method may thus be more closely related to the natural situation than powders contaminated in other studies.
- (iii) Contaminating bacteria were inactivated to some extent when the excipients were dried, but this depended on the rate of water loss from the powders. Greater bacterial inactivation was observed for contaminated powders with a low EMC, which lose physically-bound water most rapidly. Staph. aureus was more resistant to inactivation on drying than Ent. cloacae.
- (iv) Bacterial inactivation during tableting was a function of the compaction pressure applied, the organism size and shape, and the compaction mechanism of the formulation. At low pressures, plastically-deforming



excipients are most effective in killing bacteria. For lactose, which is a fracturing material, microbial inactivation was directly proportional to the applied pressure. Lubricants may reduce the extent of microbial kill, especially for fracturing materials.

- (v) There has been uncertainty about the method of microbial kill during powder compression. Ram extrusion of contaminated wet powder masses, using known and controlled levels of forces, demonstrated the particular efficiency of shearing forces in killing micro-organisms, even at low levels of compression.
- (vi) Bacteria survived in stored tablets according to both the  $A_w$ , and the hygroscopicity of the tablet formulation. Water availability to the contaminating bacteria should be described in terms of the partial vapour pressure of water ( $A_w$ ) and the water uptake characteristics of the solid to which the bacteria are attached.
- (vii) The hygroscopicity of the formulations affects bacterial survival. The behaviour of contaminating bacteria in varying conditions of water availability may thus be predicted by studying the water vapour/solid interaction. Studies of water vapour adsorption showed that the rankings of  $\Delta G$  corresponded inversely to the rankings of hygroscopicity for the excipients. A

knowledge of the thermodynamic functions of adsorption/desorption may therefore be useful in assessing the potential of a formulation for supporting viable bacteria.

- (viii) In the process of these investigations, it has been possible to quantify the complex interaction between microcrystalline cellulose and water, and to compare and contrast this with the water-sorbing nature of starch. Microcalorimetric measurements during sorption support current theories on the mechanisms of water association with celluloses.

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## NOTATION

$P$	vapour pressure
$P_o$	saturated vapour pressure
$n_1$	number of moles of solute
$n_2$	number of moles of solvent
$\nu$	number of ions generated by one mole of solute
$m$	molal concentration
$\phi$	molal osmotic coefficient
$\mu$	chemical potential
$\Delta G$	Gibb's free energy
$\oplus$	indication of a defined standard state
$K$	equilibrium constant
$R$	the gas constant
$T$	absolute temperature (degrees Kelvin)
$W$	moisture content
$W_{eq}$	equilibrium moisture content
$k$	a constant of uptake
$t$	time
$V$	volume adsorbed per gram of solid
$V_m$	monolayer volume per gram of solid
$b$	a temperature dependant constant
$c$	BET constant, related to the binding energy
$\Delta H_{mon}$	the enthalpy of adsorption of the monolayer
$\Delta H_{cond}$	the enthalpy of condensation of water
$g$	a constant related to proportion
$\Delta HB$	the enthalpy of binding
$C_g$	GAB constant, related to the free energy of binding

$i$	GAB constant, related to the solid-water interaction for layers on top of the monolayer
$q$	Clausius-Clapeyron constant
$\gamma$	liquid-air interfacial tension
$\theta$	contact angle
$V_w$	molar volume of water
$r$	capillary radius
$M_s$	total amount of water sorbed
$M_d$	total amount of water desorbed
$Y$	fraction of surface covered by a monolayer of moisture
$f$	fraction of surface with at least one normally condensed layer of moisture
$\alpha$	number of molecular layers of <i>condensed moisture</i>
$\rho$	density of water
$M$	mass of dry material
$\Delta H_{ad}$	integral enthalpy of adsorption at vapour pressure $P$
$\Delta H_{imm}$	enthalpy of immersion
$\Delta H_{imm}^P$	enthalpy of adsorption for solid equilibrated with vapour pressure $P$
$N_{ad}$	number of molecules adsorbed per unit area at vapour pressure $P$
$D$	relative density
$C$	Heckel material constant
$p$	applied pressure
$I$	particle movement constant
$N_o$	initial number of spores
$N_p$	number of spores surviving at applied pressure $p$
$d$	death rate constant
$\tau_w$	the shear stress at the die wall



- $\Delta p$       the pressure drop along the capillary
- $L$       the length of the capillary
- $[b]$       moles of water adsorbed per gram of powder